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THE INFECTIVITY OF ISOLATED INCLUSION BODIES OF FOWL-POX *

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The group of so-called filterable virus diseases is coming to occupy an increasingly important place in medical and biological literature. Included in this group is fowl-pox or epithelioma contagiosum of birds.[1] In common with many of the other virus diseases fowl-pox is characterized by the presence of inclusion bodies in the affected epithelial cells. Ever since their discovery [2] the question has been raised with regard to these bodies as to whether they represent a mere degeneration product of the cell,[3, 4] or whether, in some fashion, they actually carry the infectious agent of the disease.[5, 6]

In recent publications from this laboratory [7, 8] morphological evidence has been presented which seems to justify the opinion that the inclusion bodies represent colonies of a minute microörganism (bodies of Borrel [9]) enveloped in a membrane of lipoproteid composition which is semipermeable. The inclusions, when placed in distilled water, swell, with the formation of vacuoles in which numbers of rounded uniform bodies measuring about 0.25 micron in diameter can be seen in rapid Brownian motion. The addition of sodium chloride causes the bodies to shrink and to assume again their initial hyaline, homogeneous appearance. A crushed inclu-

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sion, suitably stained, discloses the minute bodies enclosed in a faintly staining homogeneous material which is regarded as a sort of brood capsular material for a living agent represented by the minute granules. It was in the attempt to shed some added light on the question of infectivity of the inclusion bodies that the following series of experiments was undertaken.

The characteristic lesion of spontaneous fowl-pox is a local epithelial hyperplasia. The virus proliferates locally in these eruptive cutaneous lesions and material from them is highly infectious. So far as determined an increase in virus is limited to these areas of epithelial hyperplasia. Only squamous epithelium of the skin and mucous membranes seems to be susceptible to the virus. Experimental infection is readily induced by applying active virus to any superficially injured squamous epithelial surface. If a feather be plucked from the breast of a hen and the follicle inoculated with material from an eruptive lesion a slight swelling of the follicle may be noted after three days. After seven to ten days the follicle has increased to about ten times its original size. This is shown in Fig. 1 in which the follicle "*a*" had been inoculated ten days previously. The other follicles remain normal. By carefully dissecting away the skin the swollen epithelial core of the follicle may be shelled out as shown in Fig. 2. This particular core is about 1½ mm. in diameter and about 3 mm. long. If a section be made of it the core is found to consist of concentric layers of swollen epithelial cells in each one of which may be seen one or more of the inclusion bodies (Fig. 3). The swelling of the follicle is due both to an hyperplasia of the epithelium and to a swelling of the individual cells in the stratum mucosum. The cells in this region are enlarged to several times the size of those in the stratum germinativum. The eosin-staining inclusion body is usually in the center of the cell, with a broad, non-staining zone between the inclusion and the cell membrane. The bodies may also be distinctly seen within the epithelial cells in a fresh preparation of the diseased tissue teased out in saline.

The question regarding the nature of the inclusion bodies in fowl-pox has been approached from a new angle through the discovery that, using a 1 per cent solution of trypsin in 0.2 per cent sodium bicarbonate, the cellular material of a fowl-pox lesion may be digested away completely leaving the inclusion bodies free. This process of freeing the inclusion bodies by digestion is a striking

thing to watch. If the core of epithelium just illustrated be cut into small pieces and placed on a glass slide along with a few drops of trypsin the inclusion bodies begin to show more distinctly almost immediately and, after five minutes in the solution, many of the bodies drop free from the tissue to lie like minute pearls on the surface of the slide (Fig. 4). After thirty minutes almost all of the inclusion bodies are free as shown in Fig. 5, and the amorphous mass of digested epithelium and connective tissue may be shaken about with a needle and separated completely from any remaining inclusion bodies.

The way in which the epithelial cells of a fowl-pox lesion are digested away completely, leaving the inclusion bodies unchanged, in itself suggests that these bodies represent something more than a degeneration product of the cell. As shown in Fig. 6, taken forty-five minutes after the beginning of digestion, the inclusion bodies remain intact while all that remains of the epithelium is the amorphous material seen as the groundwork of the picture.

The material which may protect the inclusion bodies from tryptic digestion is a fatty element readily demonstrable by means of special fat stains. In addition to the fat there is an albuminous component of the body.[10] The structures are elastic and may be indented with a needle only to regain their shape as soon as the needle is removed. In general the bodies are rounded or oval as shown in Fig. 7. Occasionally one may be bean-shaped or otherwise irregular. The irregularity in shape occurs usually in the larger bodies, the smaller ones being almost always rounded. In size the bodies vary from 2 or 3 microns to perhaps 50 microns in diameter. They are of relatively high density and quickly gravitate to the bottom if suspended in a physiological saline solution. They may be readily rolled about in saline on a glass slide though an occasional body may adhere to the slide and leave a small portion of its substance if broken away. The bodies are highly refractive. As shown in Fig. 7 they look, under the higher magnification of the microscope, like miniature white potatoes.

The compact nature of the inclusion bodies when freed by digestion makes them very easily manipulable for the purpose of inoculation. The trypsin may be pipetted from the surface of the bodies and be replaced with saline. This in turn may be pipetted off and the process repeated until the bodies appear to be free from débris.

After each addition of saline the bodies may be agitated in the solution since they rapidly settle to the bottom again. Those bodies which happen to strike the surface of the solution form an exception to the rule for, once on the surface, they are closely held there by surface tension. After some experimenting it was found that a 2 per cent salt solution seemed to keep the bodies slightly more firm and dense than physiological saline, so the 2 per cent solution was used exclusively for the fluid medium in our experiments.

After freeing the inclusion bodies from all cellular material an attempt was made to test their infectivity. Former attempts in this direction have been hampered by the failure to get the bodies cleaned sufficiently from their cellular surroundings. The process of digestion encouraged us in the belief that the inclusion bodies could be freed and washed clean enough to make their inoculation of value in determining whether or not the inclusion carries the infective agent of the disease. Among all of the inclusion body diseases, fowl-pox lends itself with particular readiness to this experiment because of the large size and the compactness of the inclusions. Furthermore a bird is inoculated with extreme ease since the simple operation of plucking a feather leaves an epithelium-lined follicle which is an ideal nidus for the inoculation of minute amounts of fluid.

For picking up the individual inclusion bodies a Chambers micro-dissection apparatus was found necessary. Two variations from the usual Chambers technic for isolating single bacteria by the pipette method were employed. Instead of using a hanging drop it was necessary to work with a small pool of material on the upper surface of the slide. This was because the inclusion bodies in the hanging drop fell to the dependent surface of the fluid, and once they had come under the influence of surface tension it was found impossible to remove them from the fluid. The second change found necessary was the introduction of a micro-adjustment for the plunger of the injection syringe. This syringe, when worked with the unaided hand, forced a veritable torrent of fluid through the opening of the fine capillary pipette to which it was attached. With a simple screw attachment for the plunger it was found possible to regulate perfectly the flow of fluid through the capillary opening.

The apparatus is used under the low power of the microscope with the light cut down so that the inclusion bodies show up promin-

ently on a dim field. The digesting and washing are done with the help of a dissecting microscope.

In the first inoculation experiment a small piece of infected epithelium was digested in trypsin for one and a half hours. The inclusion bodies were then washed with saline. About a hundred, as seen under the dissecting microscope, were sucked into a large pipette, transferred to another slide, and this slide was introduced under the microscope with the Chambers attachment. From five to ten inclusion bodies were sucked into a fine capillary pipette and inoculated into a breast follicle from which the feather had been plucked. Since the object of our experiment was to find whether the virus is concentrated in the inclusion or in the cellular material about it, the inoculation of inclusion bodies was controlled by the inoculation of the fluid overlying them into a follicle on the opposite side of the chicken. This fluid was by no means clear but contained numerous small particles floating in it. Consequently, when after seven days a bilateral "take" occurred on the chicken, it was thought that possibly a small inclusion body or portion of inclusion had been contained in the supernatant fluid of the control inoculation.

A refinement of technic was next attempted by picking up five or ten inclusion bodies in the capillary pipette with a minimum of fluid, transferring these to an entirely new pool of saline and from this pool picking up the inclusion bodies with a fresh pipette for inoculation into the fowl. However, this experiment too showed a "take" for both the inclusion body inoculation and for the supernatant fluid.

In the hope that a further washing of the inclusion bodies might give a supernatant fluid free from virus the bodies were next passed through two distinct pools of saline. A sample protocol of the digesting and washing process employed may be roughly diagrammed as follows, using Hen P as an illustration.

1 { Digesting pool under dissecting scope } Several hundred inclusion bodies transferred to

2 { Sorting pool under Chambers scope } 21 inclusion bodies picked up 14 delivered to

3 { First wash pool } 7 inclusion bodies picked up 4 delivered to

4 { Second wash pool } 4 inclusion bodies inoculated into anterior breast follicle, right side of Hen P.
Supernatant fluid inoculated into anterior breast follicle, left side of Hen P.

The amount of fluid sucked into the capillary pipette in the transfer from Pool 2 to Pool 3 and again from Pool 3 to Pool 4 was in each instance not over .01 cc. Pools 3 and 4 each consisted of at least 1 cc. of saline, so that the fluid carried over from Pool 2 was diluted 1 to 100 in the first transfer and again 1 to 100 in the transfer from Pool 3 to Pool 4, making a final dilution of at least 1 to 10,000 of any fluid carried over from Pool 2.

In three different experiments chickens were inoculated on the right breast with inclusion bodies washed in this fashion and on the left breast with supernatant fluid equal in amount to the fluid inoculated with the bodies. The results of these experiments were uniformly positive on the side inoculated with inclusion bodies and negative on the side inoculated with their supernatant fluid. The diagnosis of a positive result was based on a demonstration of inclusion bodies in the lesion and on its content of active virus as determined by inoculation.

Chart Showing Results of Inoculations with Washed Inclusion Bodies

Follicle Number	Number of Bodies Inoculated	Result	Control
N1	6	+	−
O1	2	+	−
P1	4	+	−
K1	1	−	−
M1	1	+	*
T1	1	−	−
T2	1	+	−
U1	1	−	−
U2	1	−	−
U3	1	−	−
V1	1	−	−
V2	1	−	−
V3	1	−	−
W1	1	+	−
W2	1	+	−
W3	1	−	−
WH1	1	+	−
WH2	1	+	−
WH3	1	+	−

* No control

The question next arose as to how many inclusion bodies must be inoculated in order to secure a "take." In the preceding experi-

ments the average number of inclusion bodies inoculated into each follicle had been four. A series of sixteen follicles was next inoculated with one inclusion body in each follicle. As shown in the chart seven of these inoculations showed a "take" while nine were negative. In no instance, however, was a single control inoculation positive.

A probable explanation of the failure to secure positive results in a larger percentage of the single inclusion body inoculations seemed to lie in the difficulty experienced in delivering a single body from the pipette into the follicle. As graphically illustrated for Hen P only fourteen of the twenty-one inclusion bodies picked up could be delivered into the first wash pool and only four out of seven could be delivered into the second wash pool. This example is characteristic of our experience in all of the attempts to transfer the inclusion bodies from one pool to another and indicates a propensity of the bodies for sticking to the wall of the pipette. Considering this difficulty the low percentage of "takes" is not remarkable. There was no gradation in the size or in the time of appearance of the "takes," those which appeared at all coming on just as quickly as those which had been inoculated with four or five inclusion bodies.

The lesion caused by the inoculation of one inclusion body is shown in Fig. 8. Each of the two swollen follicles was inoculated seven days previously with a single inclusion body. The swollen follicles are 2 to 3 mm. in length and contain several hundred thousand inclusion bodies similar to the single inclusion body with which each was originally inoculated. The anterior one of these two follicles was removed and digested so as to free the bodies. The digestion mixture was then smeared on the plucked and scarified breast of a chicken. The lesion resulting after seven days is shown in Fig. 9. The massive "take" in the plucked follicles and along the lines of scarification is, then, the result in two weeks time of the inoculation of a single inclusion body.

Discussion

The rapid development of a lesion following inoculation with a single inclusion body we have interpreted as being due to the inoculation not of a single microörganism but of a large number enclosed within the inclusion. Special stains of crushed inclusions show by

count hundreds of definite, rounded objects about 0.25 micron in diameter within each body.[7] These minute objects are believed to represent the actual virus of fowl-pox. Consequently, in inoculating a single inclusion body, one is probably inoculating an entire colony of virus bound up in its fatty capsule.

Working with the digested material from a fowl-pox lesion gives one a very definite impression as to the character of the inclusion bodies. The way in which the bodies resist tryptic digestion marks them as something more substantial than the cell which contains them. The way in which the bodies can be washed clean and inoculated to give a "take," while their supernatant fluid remains sterile, indicates that the inclusion bodies actually carry the infectious agent of the disease. The way in which a single inclusion body, when inoculated, produces in two weeks time a lesion containing millions of similar bodies indicates that the inclusion bodies of fowl-pox contain and are caused by a living, growing virus. So certain have we become that the inclusions carry a virus that the bodies are now spoken of with confidence as *virus* bodies rather than inclusion bodies.

The results of these experiments afford additional evidence that the typical lesion of fowl-pox is induced through an invasion of epithelial cells by the virus and its intracellular proliferation locally within the lesion.

Summary

1. The inclusion bodies of fowl-pox are composed of hundreds of minute bodies enclosed in a fatty capsule.

2. The hyperplastic epithelium of the lesion of fowl-pox, when subjected to tryptic digestion, liberates the intact inclusion bodies while the epithelial cells undergo complete digestion.

3. A single inclusion body, when washed with saline and inoculated into the skin of the hen, has produced a typical fowl-pox lesion containing the characteristic inclusions. The fluid in which the inclusion body is finally suspended is innocuous.

4. The inclusion bodies of fowl-pox are interpreted as being true *virus* bodies, *i.e.*, minute colonies of the etiological agent of the disease.

5. The proliferation of the virus is accordingly largely, if not entirely, intracellular.

REFERENCES

1. Marx, E., and Sticker, A. Untersuchungen über das Epithelioma contagiosum des Geflügels. *Deutsche med. Wchnschr.*, 1902, xxviii, 893.
2. Bollinger, O. Ueber Epithelioma contagiosum beim Haushuhn und die sogenannten Pocken des Geflügels. *Virchows Arch. f. path. Anat.*, 1873, lviii, 349.
3. Sanfelice, F. Untersuchungen über das Epithelioma contagiosum der Tauben. *Ztschr. f. Hyg. u. Infectionskrankh.*, 1913, lxxvi, 257.
4. Ludford, R. J., and Findlay, G. M. The ultra-microscopic viruses: II. The cytology of fowl-pox. *Brit. J. Exper. Path.*, 1926, vii, 256.
5. v. Prowazek, S., and Aragao, H. B. Weitere Untersuchungen über Chlamydozoen. *München med. Wchnschr.*, 1909, lvi, 645.
6. Lipschütz, B. Untersuchungen über Epithelioma contagiosum der Vögel. *Centralb. f. Bakteriol.* Abt. I, Orig., 1908, xlvi, 609.
7. Goodpasture, E. W. Rivers' Filterable Viruses, Baltimore. Williams and Wilkins, 1928, 235–277.
8. Goodpasture, E. W. The pathology of certain virus diseases. *South. M. J.*, 1928, xxi, 535.
9. Borrel, A. Sur les inclusions de l'épithélioma contagieux des oiseaux. *Compt. rend. Soc. de Biol.*, 1904, lvii, 642.
10. Michaelis, L. Mikroskopische Untersuchungen über die Taubenpocke. *Ztschr. f. Krebsforsch.*, 1903, i, 105.

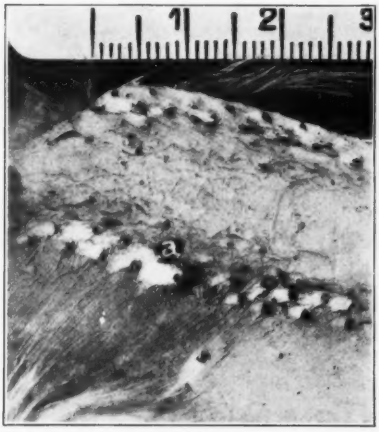
DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Swollen feather follicle at "*a*," ten days after inoculation with a single inclusion body of fowl-pox.

FIG. 2. Epithelial core of inoculated feather follicle. This particular core was removed from the anterior follicle illustrated in Fig. 8.

FIG. 3. Section through feather follicle removed ten days after inoculation with fowl-pox. The dark bodies within the swollen epithelial cells are the inclusion bodies. Hematoxylin and eosin. × 80.



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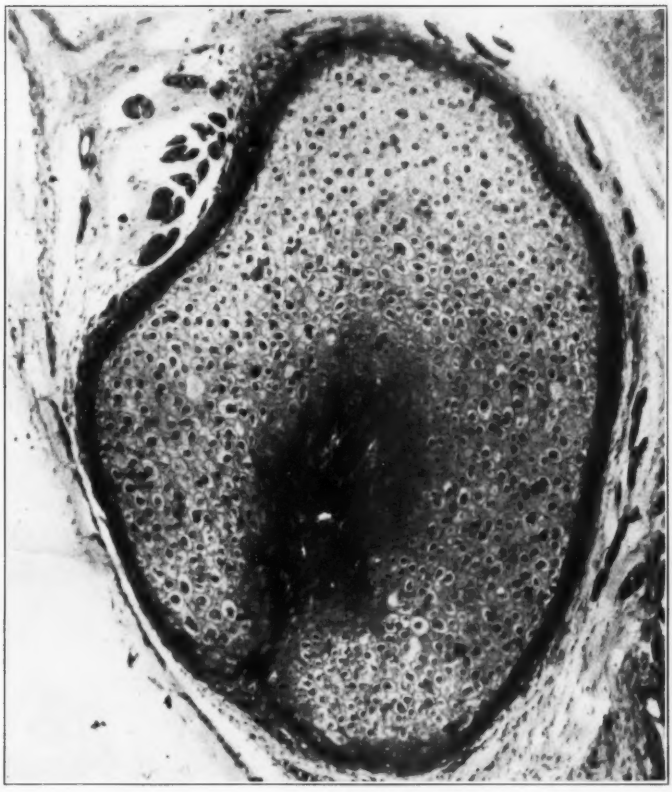
2



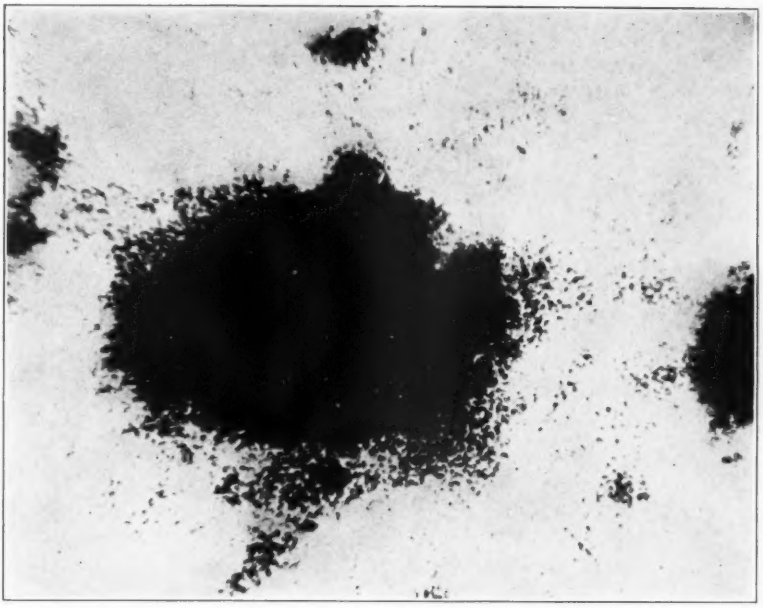
3

Woodruff and Goodpasture Infectivity of Inclusion Bodies of Fowl-Pox

PLATE 2

FIG. 4. Mass of infected epithelial cells in the process of digestion. Some of the freed inclusion bodies may be seen on the right-hand edge of the epithelial mass. × 47.

FIG. 5. Inclusion bodies thirty minutes after the beginning of digestion. × 160.



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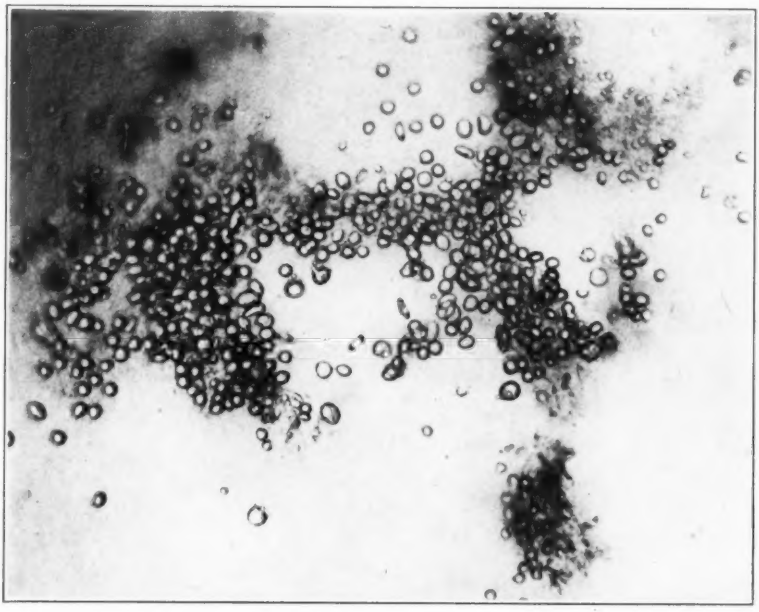

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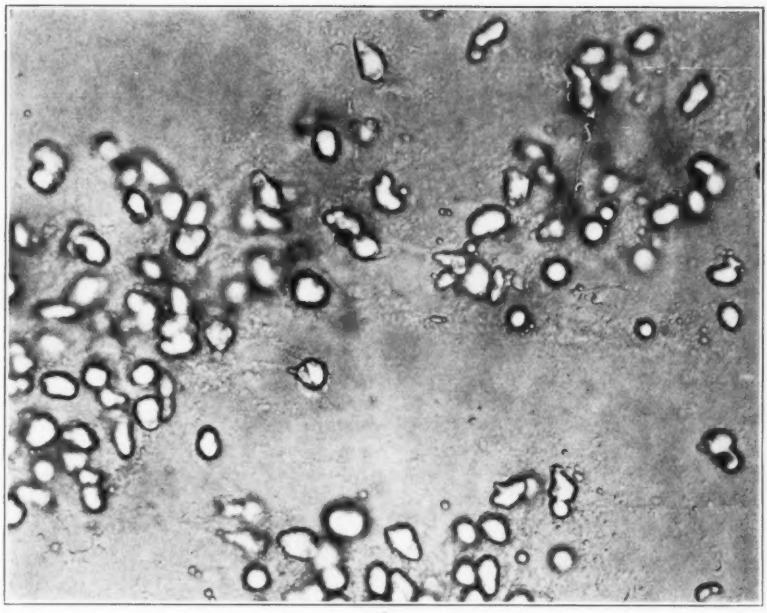
PLATE 3

FIG. 6. Inclusion bodies forty-five minutes after the beginning of digestion. Amorphous material forming the groundwork of the picture represents the digested epithelium. × 330.

FIG. 7. High magnification of inclusion bodies completely freed by digestion. × 1000.



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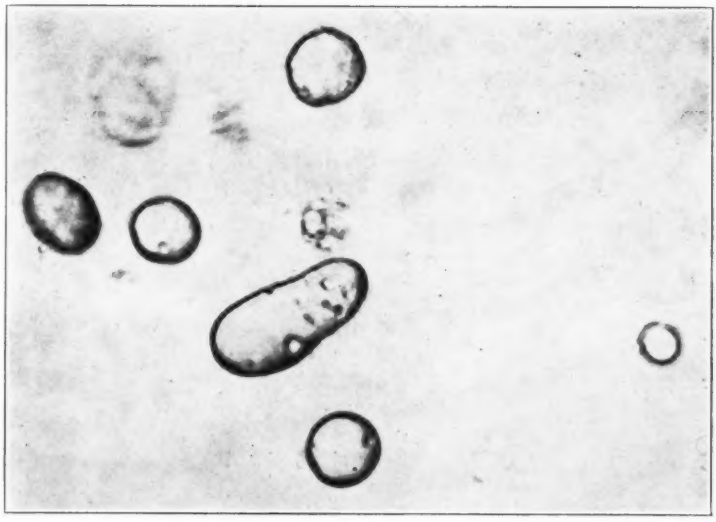

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PLATE 4

FIG. 8. Two feather follicles each of which had been inoculated seven days previously with a single inclusion body.

FIG. 9. Plucked and scarified breast of hen seven days after inoculation with inclusion bodies from the anterior follicle illustrated in Fig. 8.

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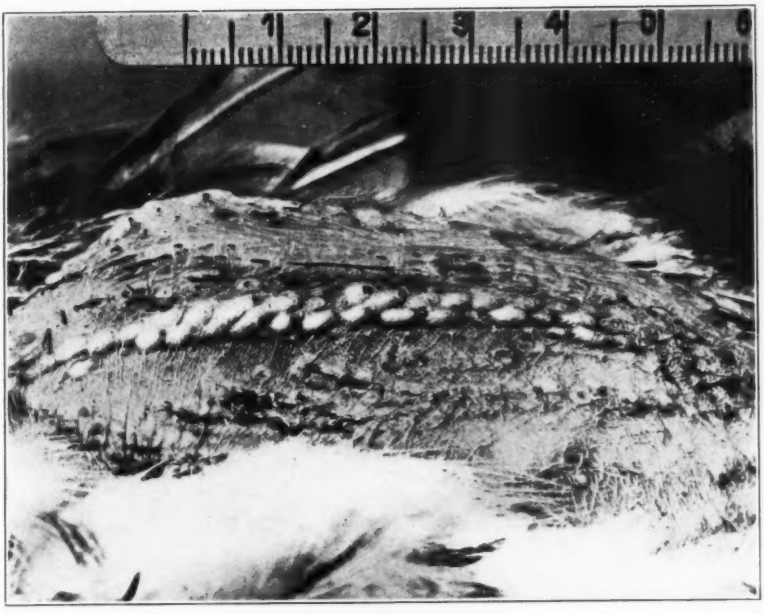

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A CASE OF EXTENSIVE BILATERAL PROGRESSIVE THROMBOSIS OF THE SMALLER BRANCHES OF THE PULMONARY ARTERIES *

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In order to emphasize the importance of disease in the pulmonary arteries Posselt [1] in 1909 published an extensive and comprehensive review of the lesions in these vessels that had been reported over a long period of years. He divided the lesions in the pulmonary arteries into groups depending upon the nature of the lesion, whether primary or secondary, whether associated with acquired or congenital cardiac disease and whether there was disease in the other arteries of the body. Among the various lesions described Posselt refers to eighteen reports of cases of thrombosis with endarteritis of the pulmonary arteries. In some of these cases the thrombosis was extensive and occurred sometimes in the smaller branches of the pulmonary arteries but the distribution of the lesions and the clinical picture of none were sufficiently similar to the case here reported to identify any of them as the same disease. For a very complete review of the literature up to 1909 one is referred to this article.

The causes assigned to the production of the various vascular lesions in the pulmonary arteries described in Posselt's review were often somewhat obscure although special disorders seemed to be associated with certain of the vascular changes. Thus conditions which caused an increase of pressure in the pulmonary circulation such as passive congestion from left-sided cardiac disease, emphysema, disease of the pleura and pulmonary tuberculosis were blamed for lesions in the pulmonary arteries. In like manner syphilis, the acute infections, especially smallpox, and congenital weakness of the blood vessels were considered as etiological factors. Kitamura [2] thought that plethora from excessive beer drinking was a cause of lesions in the pulmonary arteries and Emery [3] the misuse of alcohol. For the cases with thrombosis in the pulmonary arteries no special etiological factor was mentioned as a probable cause.

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Most of these cases of disease of the pulmonary arteries showed hypertrophy of the right side of the heart, in some instances even very extensive hypertrophy. Most of them also showed cardiac failure with edema or dropsy in some parts of the body.

Since that very complete review by Posselt in 1909 there have appeared in the literature a few reports of disease of the pulmonary arteries but on the whole the subject does not seem to have received much attention. Most of the articles have laid emphasis on the disturbance in the heart that pulmonary vascular lesions may cause. Sanders [4] in 1909 reported a case of sclerosis of the smaller branches of the pulmonary artery without offering any reason for the lesions. Arrillaga [5] in 1913 published a monograph on the clinical syndrome of chronic cyanosis, dyspnea and erythremia described by Ayerza in 1901 as due to sclerosis of the pulmonary artery which has been spoken of as Ayerza's disease. These lesions were in the larger branches and at the origin of the pulmonary artery. The cause of this sclerosis was not made clear. Warthin [6] in 1919 ascribes this arterial lesion to syphilis and there is apparently no doubt that syphilis does produce changes in this artery. Moschcowitz [7] in 1927 claimed that the lesions of Ayerza's disease were not due to syphilis but were due to increased pressure in the lesser or pulmonary circulation.

In 1915 Veale and Coombs [8] described gross sclerosis in the pulmonary arteries which they spoke of as primary because no apparent cause was found. In 1914 Schütte [9] reported a case in which the lesions in the pulmonary arteries were in the smaller branches and were detected only under the microscope. He offered no cause for the lesions but the patient was 74 years old and the lesions might have been the result of age, although the pictures suggest that they might have been the result of organization of thrombi. Also in 1916 Hart [10] reported two cases of sclerosis of the pulmonary arteries without apparent cause.

Krutzsch [11] in 1920 reported on the lesions that may occur in the pulmonary arteries and of special interest was his report of a case with thrombi in the small arteries and capillaries of the lungs associated with a wide dissemination of cancer cells. Gamna [12] in 1921 pointed out that sclerosis of the pulmonary arteries occurred in congenital cardiovascular malformations and chronic pleuropulmonary disorders. The sclerosis in these conditions usually involved the

larger branches. He also claimed that a primary sclerosis occurred in the smaller branches of the pulmonary arteries without any apparent cause. The lesions often simulated endarteritis obliterans. Eppinger and Wagner [13] in 1920 called attention to lesions in the smaller branches of the pulmonary arteries without apparent lesions in the larger branches so that nothing was suspected on gross examination. In their cases there were often thrombi in the small arteries, and they resembled in many ways the one here reported. They suggested that underlying the thrombi in the arteries there were demonstrable lesions of the arterial walls. They discussed the various causes of vascular lesions without reaching any definite conclusions and pointed out that many of the theories regarding the cause of these vascular lesions did not fit their cases. In 1927 Clark, Coombs, Hudfield and Todd [14] reported five cases of disease in the pulmonary arteries in one of which there was an obliterating endarteritis which they thought due to syphilis and one case with thickening and nodules in the branches of the pulmonary arterial tree that were only from five to one millimeters in diameter. The cause for the lesions in these small arteries was not apparent.

It is quite obvious, therefore, that various lesions occur in the pulmonary arteries and that these lesions may occur in the larger or smaller branches alone, or in both. Syphilis undoubtedly causes some of the lesions. Other lesions similar to arteriosclerosis also occur without the etiology being any more apparent than it is in arteriosclerosis elsewhere in the body. In this group increase in the intra-pulmonary pressure, diseases of the lungs or pleura and acute infections are among the causes blamed for the arterial lesions. Congenital defects are also considered as a cause of some of the vascular lesions. In addition a few instances of thrombotic lesions with obscure etiology in the smaller pulmonary arteries have been reported.

The following case, in which interesting lesions were found in the pulmonary arteries, was observed clinically and came to autopsy.

Case Report

Clinical Record: Peter Bent Brigham Hospital Number 51761.

The patient was a single, white woman, aged 38 years who entered the Peter Bent Brigham Hospital on April 15, 1926, and died on April 24, 1926. Her chief complaint was shortness of breath.

The family history was unimportant. In childhood she was ill with chickenpox, measles, mumps, diphtheria, whooping cough and possibly typhoid fever.

All her life she had been subject to frequent "colds" and had been somewhat deaf for twenty years. Five years before entry her tonsils were removed.

The exact time of the onset of her final illness was not clear, but she claimed that she was perfectly well up to about six months before admission to the Brigham Hospital. At that time while having a severe "cold" in the head she noticed marked shortness of breath while on a mountain climbing trip. This soon disappeared. About four months before admission, associated with another cold in the head, shortness of breath was again marked and there was also a sharp pain under the left breast upon deep breathing or coughing for three or four days. Following this upset her shortness of breath did not entirely disappear and similar symptoms occurred off and on until she entered the Mt. Sinai Hospital in New York under the care of Dr. Marcus A. Rothschild about the middle of January, 1926.

Dr. Rothschild has kindly sent me a summary of his findings and his opinion in regard to the patient. He finally decided upon neurocirculatory asthenia on a post-infectious basis as the best diagnosis he could make, although he was by no means satisfied with this diagnosis. An X-ray of the chest at that time showed the lungs to be clear and the left ventricle of the heart somewhat rounded but without definite enlargement of the heart. An electrocardiogram was normal except for an inversion of the T wave in the third lead.

Following her discharge from the Mt. Sinai Hospital she took a short rest and then returned to her work in New York as manager of a tea room.

About six weeks before admission to the Brigham Hospital with another "cold" the shortness of breath returned and also a pain in the left side of the chest. She gave up work and went to visit relatives but the shortness of breath became so pronounced that she was unable to walk more than a few steps without becoming markedly short of breath. Also her appetite became quite poor and she vomited several times. On three occasions a short time before admission she practically fainted on rather slight provocation. During these months she had lost from 15 to 20 pounds in weight.

Physical examination upon entrance at the Brigham Hospital was essentially negative except that upon such slight exertion as talking or moving in bed she became cyanotic and exceedingly short of breath. She was able, however, to lie flat comfortably. Her blood pressure was 92 systolic and 68 diastolic. Her hemoglobin by the Dare method was 80 per cent; red blood count 5,630,000; white blood count 14,350. The differential leukocyte count showed polymorphonuclear neutrophiles 70 per cent; lymphocytes 23 per cent; large mononuclears 6 per cent; and eosinophiles 1 per cent. In the smear the red blood cells appeared normal. Subsequent examinations showed that the leukocytosis increased slightly. The urine was essentially negative on several examinations except for a moderate number of leukocytes, the origin of which was not investigated. The stools were normal and the blood Wassermann reaction negative. A basal metabolism study on April 17 showed +5 per cent. Her vital capacity on April 20 was only 53 per cent of her theoretical normal. One physician who examined her thought that the right side of the chest moved more freely than the left and that there was slight dullness and a few crackling rales in the upper left front. The house physician made the preliminary diagnosis of neurocirculatory asthenia.

On March 31, 1926, Dr. L. B. Morrison of Boston reported upon the X-ray study of her lungs by stereoscopic plates. He found increased fibrosis at the hilum of both lungs and quite a bit of fibrosis at the cardiohepatic angle. He

also found some thickened pleura at the very peak of the left diaphragm without any free fluid. He thought that there had been some subacute inflammatory process at that point which had caused a peribronchial thickening and a thickened pleura and that it was subsiding. There was no evidence of tuberculosis.

On April 20, 1926, a seven-foot film of the heart taken at the Brigham Hospital showed a definite enlargement in the region of the left ventricle. The internal diameter of the chest was 22.5 cm.; the right border of the heart 4.4 cm. and the left border 9.6 cm. from the midsternal line. The shadow of the great vessels above the heart was 5.5 cm. wide.

During her stay in the hospital her temperature was not elevated. Her pulse rate was with few exceptions between 100 and 110 per minute and it did not become appreciably faster as death approached. Her respirations at rest ranged from 20 to 30 per minute.

An electrocardiogram showed a right ventricular preponderance with an inverted T wave in both the second and third leads. During her stay of nine days in the Brigham Hospital the shortness of breath upon exertion, no matter how slight, became more marked. She became gradually more cyanotic upon exertion and even at rest, and finally somewhat orthopnoeic. She also developed exhausting coughing spells. Her pulse became gradually weaker in volume. On the morning of her death as she was markedly cyanotic and struggling for breath one of the attending physicians remarked that had the symptoms developed suddenly it would be the typical picture of an embolus in the pulmonary artery.

Autopsy Report

A postmortem examination was made eight hours after death without opening the skull. From the heart's blood a streptococcus hemolyticus was obtained in culture but as no signs of its activity were seen in the lungs or other organs its presence was not considered of importance. Although the important findings were in the lungs and heart a few points of interest were found in other parts of the body and will be mentioned first. The organs that are not mentioned were essentially normal both in the gross and under the microscope.

Slight passive congestion was found in the liver, spleen and kidneys. In addition, in the liver there were some small areas of early focal necrosis. Also in the liver there were a few small nodules containing giant cells in some of which were small stellate bodies. Similar lesions were also present in the lungs and will be described in more detail below. In the uterine wall there were a few small leiomyomata and about the external os of the cervix there was a superficial erosion with microscopic cysts in the glands beneath. Furthermore in the left uterine vessels and their contributaries there were ante mortem clots which in some instances completely occluded the

vessels. Also in one section of the vagina for microscopic study a large adjacent vein contained a thrombus without evidence of organization or infection about it. The aorta showed only slight evidence of arteriosclerotic changes and the smaller arteries in the various organs of the body seemed in excellent condition except for those in which thrombi were present.

Heart: The heart weighed 280 gm. It seemed somewhat distended and rather large for the weight and after being severed from the great vessels collapsed, especially upon the right side. The valves and coronary arteries were not remarkable. No evidences of mural thrombi were found. The myocardium was somewhat softer in consistence than normal and a little pale but there was no gross evidence of fibrosis. The wall of the right ventricle was hypertrophied, especially toward the base where it measured 1 cm. in thickness tapering to 0.3 cm. in thickness toward the apex. In the right ventricle the trabeculae carneae were much hypertrophied. Microscopic examination of the heart was also essentially negative except for a section of the auricle which showed a thrombus which apparently was an ante mortem formation but quite recent as there was no evidence of beginning organization. This was apparently overlooked in the gross inspection of the auricles although they were carefully searched for mural thrombi.

Upon opening the pulmonary artery within the pericardial cavity only fluid blood was found in the larger branches and nothing remarkable was noted about the vessels.

Lungs: The lungs were normal in shape and pink in color except for the extreme posterior borders where they had a dark red almost purplish tinge. This darkening of the posterior margins covered a smaller area than ordinarily noted. The right lung weighed 300 gm.; the left 250 gm.

The left lung was crepitant throughout except for two small areas, one on the lower sharp border of each lobe. Here on the surface of the lung there were sharply demarcated dense white areas each triangular in shape, the height of the triangle being about 1.5 cm. and the base resting at the sharp border of the lung. This tissue was very hard in consistence and had almost a cartilaginous feel. It extended between both surfaces of the lung and the edge of the tissue occupied the sharp border. The surface here was somewhat softer than the remainder of the lung. Surrounding these triangular white areas

there was a region of crepitant dark red lung which merged insensibly into the more nearly normal pink lung about it. On section of this portion of the lung the white appearance of the surface extended completely through the central part of these areas taken to be old infarcts and about them was a border of congested firm lung tissue about 0.5 cm. in width. Sections elsewhere in the left lung showed only crepitant grayish pink tissue which however seemed to have a slight brownish tinge. It had a somewhat more rubbery feel than normal lung but was crepitant throughout. No exudate could be expressed from this lung.

In the right lung five or six infarcted areas were found in similar locations to the two in the left lung, that is along the sharp borders of all three lobes. Two of these had a similar appearance to the two in the left lung, that is the central portion was dense, almost cartilaginous. The others varied from this picture to one suggesting a very recent infarct. This latter area was raised somewhat above the surface of the surrounding lung and was dark red in color and at its border; both on the surface and on cut section it had a fairly well demarcated edge. Intermediate between these two extremes the other infarcts had a grayer center than the firm one and this central portion was softer although still quite firm. About each of them was a rim of firm congested crepitant lung tissue. Elsewhere the right lung showed a similar appearance on section to the left lung.

Dissection of the bronchi in both lungs revealed a slightly congested mucosa to which there was attached a small amount of brownish mucoid material. Dissection of the pulmonary veins revealed nothing unusual. The lesions about to be discussed were present in both lungs but much more frequent in the right. Upon opening the pulmonary arteries nothing unusual was found in the first and second order of branches peripheral to them. After the dissection of the branches of the third and fourth order was attempted practically all of them were found completely occluded by thrombi. These thrombi varied in size from small pin-head-sized crumbly grayish pink masses firmly attached to the wall to similar appearing masses 1 or 2 cm. in length and filling the whole lumen of the vessel. Propagated from these older thrombi in the direction of the root of the lung were adherent long dark red softer thrombi. In many cases the thrombi when pulled off were seen to be extending into some of the small pinhead size ultimate branches of the vessels, leading to the parenchyma

of the lung. When these comparatively fresh thrombi were torn off from the walls of the arteries they left rough surfaces which in some places were slightly ulcerated. In addition to these fresh thrombi a few very firm, very dense yellowish white thrombi were found firmly attached to the wall and almost compeltely filling the lumen. These latter thrombi could not be very readily crushed in the fingers, although most of the grayish pink ones could be so crushed. Other portions of the lining of the pulmonary arteries were somewhat roughened and had a yellowish white appearance although no thrombi could be rubbed from the surface. This was most marked in the smaller branches and in several places a small ring of this roughening and thickening was found surrounding one of these ultimate pin-point branches.

Microscopic Examination

For microscopic study sections were made from twenty-three blocks of the lung tissue. In all there was a very considerable increase of connective tissue in the alveolar walls and about blood vessels and bronchi. In some regions the alveolar walls were almost avascular. The alveoli throughout the lungs contained numerous mononuclear cells, most of which contained carbon pigment. Adjacent to blood vessels, bronchi, pleura and interlobular septa were tubercle-like lesions composed of mononuclear cells and giant cells (Figs. 1 and 2). Most of these lesions contained numerous lymphoid and plasma cells and some showed beginning fibrosis. In the giant cells were found the stellate inclusions described by Wolbach [15] in 1911, and the histology and distribution of the lesions in the lung were in general identical with those described by him. The lesions in the liver with giant cells mentioned above probably represent the same process.

The infarctions noted in the gross showed varying degrees of repair. Some of them consisted essentially of rather dense cicatricial tissue but the majority showed necrotic centers, preserving roughly the architecture of the lung parenchyma and surrounded by dense fibrous tissue. There were a few recent infarctions with hemorrhagic peripheral zones undergoing organization. None of the infarctions showed evidence of suppuration.

The important pathology of the lung concerns the blood vessels. Many of the smallest recognizable arteries showed an extraordinary

thickening of their walls and were composed of a dense connective tissue with a very narrow lumen lined with swollen endothelial cells. Here and there these vessels showed acute change, on the whole reminiscent of the lesions found in the glomerular arterioles in chronic nephritis accompanied by hypertension (Figs. 3 and 4). The smooth muscle layer was intact but the media greatly thickened and sparsely infiltrated with mononuclear cells (macrophages). The lumen in most instances was almost completely obliterated by compression and lined with swollen endothelial cells. The thickened intima was characterized by a lightly staining collagen and by the presence of fairly well preserved red blood corpuscles, as if both the endothelium and connective tissue had become permeable to particulate matter.

These acute lesions were comparatively few in number and concerned vessels of the diameter of 0.03 mm. to 0.05 mm. Subsequent sections in the vascular lesions were difficult to follow. It was quite evident that complete occlusion of some small arteries had resulted. In the majority of instances the repair had been accomplished by a new deposit of collagen producing concentric or eccentric thickening of the blood vessel wall and throughout the sections studied there were found small arteries showing early and late cicatricial thickening of the intima (Fig. 5). In larger arteries there had evidently been organization of thrombi inasmuch as many were found containing several endothelial-lined channels. In arteries regarded as showing the oldest lesions thickened walls were frequently found to contain cells resembling in all respects smooth muscle cells such as are found frequently in old canalized thrombi (Fig. 6). In arteries of larger caliber, of dimensions in the neighborhood of 1 mm. to 1.3 mm. there were found completely organized and canalized thrombi. The channels were lined with endothelium and backed up by smooth muscle cells; these represented the late stage of repair (Fig. 7). Such canalized arteries on the whole show a practically normal media and a normal distribution of elastic tissue. Fresh thrombi and thrombi with early organization were found only in arteries of considerable size, those with a diameter of 1.5 mm. and over (Fig. 8).

In these larger arteries the histology gave no clue to the causation of the thrombi. On the other hand, the microscopic findings supported the conclusions made at the time of the postmortem that these fresh thrombi were propagated from the more distal organized

and canalized thrombi. In none of the sections were arteries found with unusual degrees of the arteriosclerotic or atheromatous processes. Only in arteries in close proximity to infarctions were acute changes simulating the early stages of atheromatosis found.

Discussion

While in the study of these lesions many details were noted in the histology of the blood vessel lesions, no completely satisfactory selection of lesions could be made in the attempt to work out the sequences which gave rise to the predominating pathological condition, *viz.*, a propagating thrombosis with organization and canalization.

It seems warrantable to conclude that the acute lesion, consisting in swelling and cellular infiltration of the vessel wall represents an early if not the initial damage. The infiltrating cells for the greater part were mononuclear cells (macrophages). Polymorphonuclear leukocytes were practically absent. Eosinophiles and mast cells were occasionally present. The presence of mononuclear cells together with the swollen endothelium permits the consideration of the possibility of an infectious agent as causative factor.

A wholly warrantable conclusion is that the oldest lesions as well as the most recent are to be found in the minute arteries and that the thrombosis traveled by centripetal progagation. The explanation of the continued propagation of thrombi over the long period indicated by the clinical history and by the histological findings presents a problem not solvable by the histological findings, because the type of lesion seen in the smallest arteries is not present in the larger arteries with recent thrombi, nor is there anything in the appearance of the organized and canalized thrombi to account for the continued propagation.

An hypothesis which may be entertained is that the lungs in this case had been the seat of an extensive interstitial pneumonitis. This hypothesis is suggested by the generalized fibrosis and by the tubercle-like lesions adjacent to bronchi, blood vessels and connective tissue structures of the lung, all regions of lymphatic channels. In fact it is quite evident that many of the smaller tubercle-like lesions have arisen in lymphatics. Whether or not the initial vascular damage was done during the period of interstitial pneumonitis cannot be solved, but it would seem reasonable to accept this explanation

rather than to assume the presence of an unknown infectious agent of unique behavior.

On the whole, the closest analagous vascular pathology is to be be found in the disease known as thrombo-angiitis obliterans. Aside from the distribution of the lesions, the similarity of the process to the present case seems evident. At least there is in both conditions centripetally propagated thrombosis of arteries accompanied by organization and canalization. The pathology of the arteries in this case is more nearly expressed by the term endarteritis or arteritis than by arteriosclerosis or atheromatosis.

It is not evident whether the thrombosis in the uterine vessels and the microscopic thrombus found in the auricle were due to the same process as the lesions in the pulmonary arteries. The lesions in the pulmonary arteries were evidently much older.

In this case it is interesting to note that with these multiple small infarctions of all ages in the lungs very little evidence of them was found upon X-ray examination. It is also interesting to speculate whether the so-called fresh "colds" and periods of shortness of breath represented periodic advancement of the thrombus formation, or recurrence of the acute arterial lesions. In the cases cited from the literature, even those by Eppinger and Wagner which strongly suggested the lesions found in this case, the outstanding symptoms usually were cardiac failure. In this case the heart muscle had not given way to the extent of the production of edema and pronounced passive congestion, but already evidence of hypertrophy of the right ventricle had appeared and it is possible that the lack of symptoms of cardiac failure could be explained by the fact that the patient died early in the course of the disease.

The lesions in this case are certainly different from ordinary arteriosclerosis, also from lesions due to syphilis and also from some of the cases of thrombosis of the pulmonary arteries that have been reported. The lesions may be similar to those in a few of the cases with thrombosis of the pulmonary arteries and cardiac failure that have been reported. Possibly the case may represent a unique lesion of the pulmonary arteries.

Summary

This case clinically during life presented symptoms of unexplained shortness of breath and cyanosis upon exertion gradually increasing

over a period of months. The cause for it became apparent at autopsy as due to thrombosis of the smaller branches of the pulmonary arteries with resulting infarctions and injury to lung tissue.

This thrombosis began in the smallest branches of the pulmonary arteries and propagated centripetally toward the larger branches.

The cause for the beginning of the thrombosis or its tendency to propagate was not apparent.

In the walls of some of the smallest branches of the pulmonary arteries were slight acute lesions for which the cause was not apparent.

The relation of the tubercle-like lesions in the lung to the vascular lesions is unsettled.

Note: For the photomicrographs and many helpful suggestions I am indebted to Professor S. Burt Wolbach.

REFERENCES

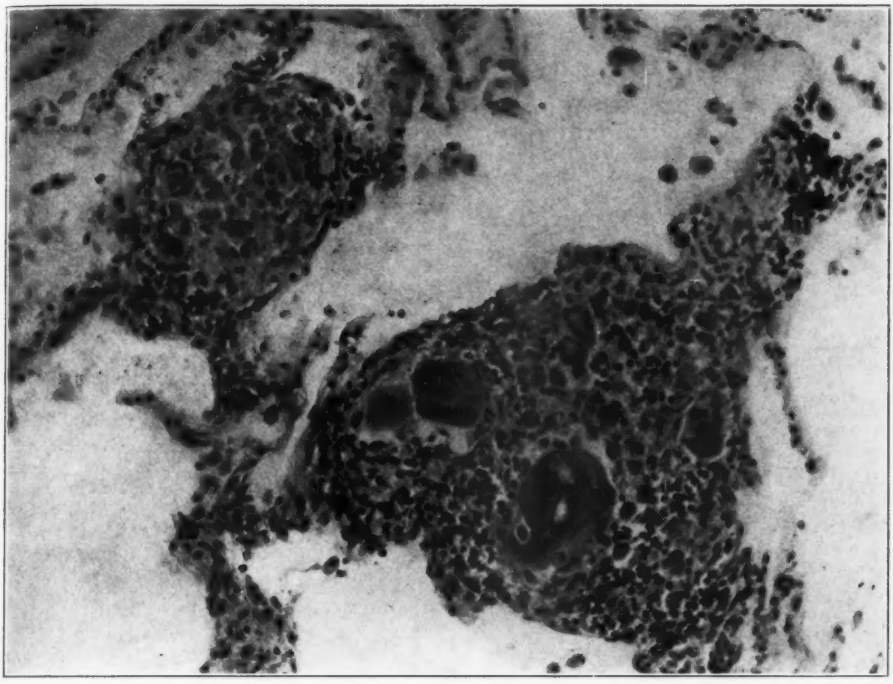
1. Posselt, A. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1909, xiii, 1 abt., 298.
2. Kitamura, S. *Ztschr. f. klin. Med.*, 1908, lxv, 14.
3. Emery. Cout. à l'étude de l'atherome pulmonaire. These, Paris, 1889.
4. Sanders, W. E. *Arch. Int. Med.*, 1909, iii, 257.
5. Arrillaga, F. C. Monograph. Esclerous Secundaria de la Arteria Pulmonar, 1913.
6. Warthin, A. S. *Am. J. Syph.*, 1917, i, 693; *Contrib. to Med. & Biol. Research*, 1919, ii, 1042.
7. Moschcowitz, E. *Am. J. M. Sc.*, 1927, clxxiv, 388.
8. Veale, P. J., and Coombs, C. *Brit. J. Child Dis.*, 1915, xiii, 72.
9. Schütte, H. *Centrallbl. f. allg. Pathol. u. path. Anat.*, 1914, xxv, 483.
10. Hart, C. *Berl. Klin., Wchnschr.*, 1916, liii, 304.
11. Krutzsch. G. *Frankfurt. Ztschr. f. Path.*, 1920, xxiii, 247.
12. Gamna, C. Abstract in *M. Science*, 1922, vi, 507.
13. Eppinger, H., and Wagner, R. *Wein. Arch. f. inn Med.*, 1920, i, 83.
14. Clark, R. C., Coombs, C. F., Hudfield, G., and Todd, A. T. *Quart. J. Med.*, 1927, xxi, 51.
15. Wolbach, S. B. *J. Med. Res.*, 1911, xxiv, 243.

DESCRIPTION OF PLATES

PLATE 5

FIG. 1. Tubercle-like lesion in the lung showing giant cells. × 210.

FIG. 2. Tubercle-like lesion in the lung showing giant cells. × 210.



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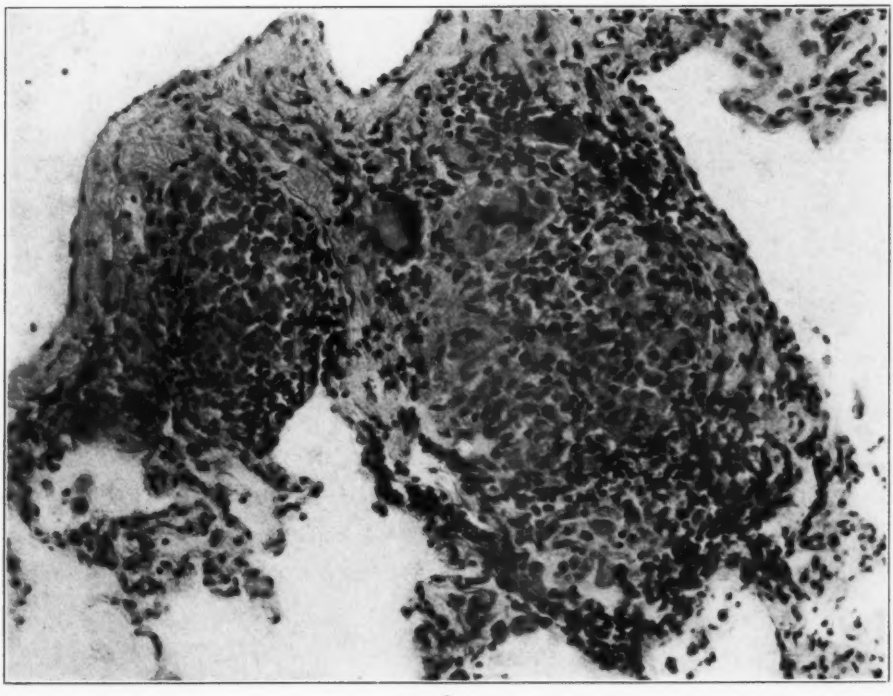

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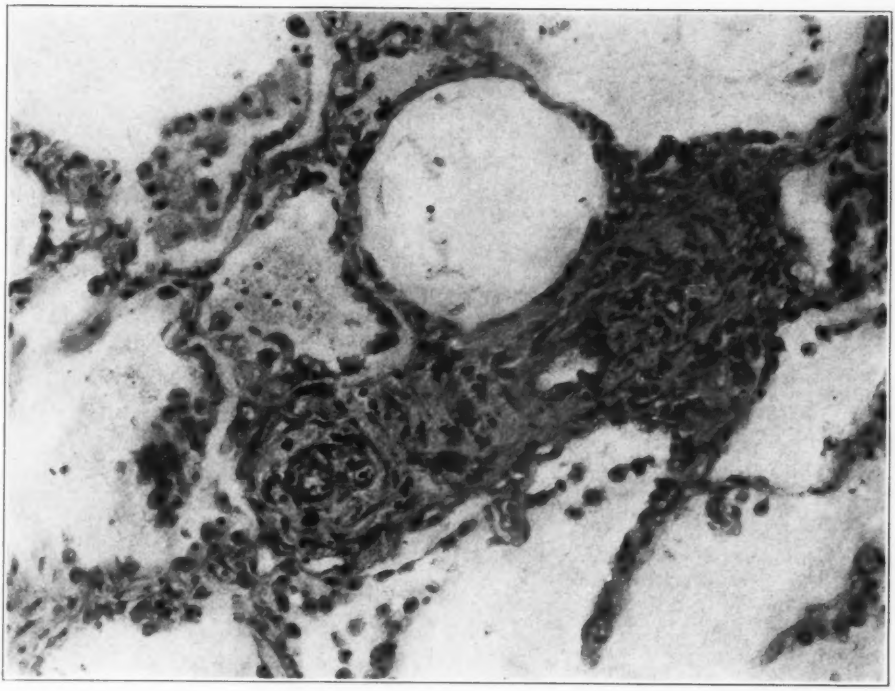
Frothingham Thrombosis of Branches of Pulmonary Arteries

Plate 6

Fig. 3. Acute vascular lesion showing thickening of the vessel wall with swelling of the endothelium and invasion with mononuclear phagocytes. × 210.

Fig. 4. Acute vascular lesion showing thickening of the vessel wall with swelling of the endothelium and invasion with mononuclear phagocytes. × 210.



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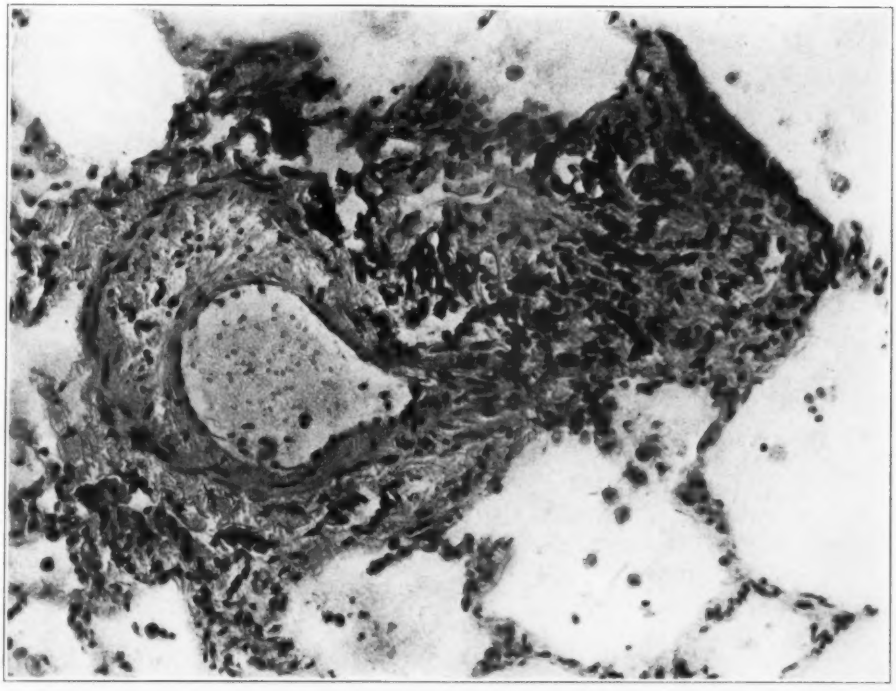

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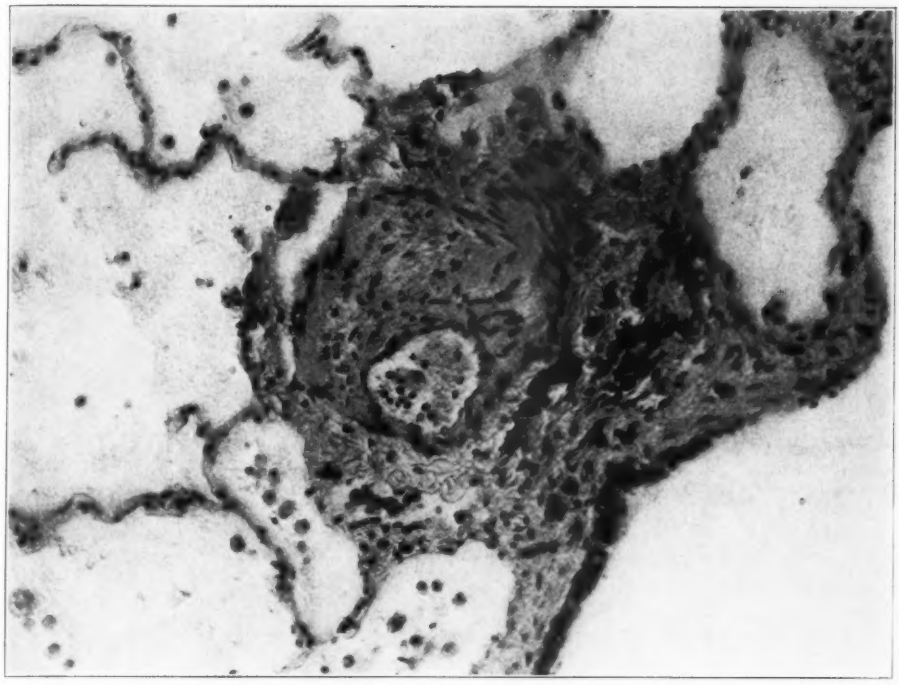
Frothingham Thrombosis of Branches of Pulmonary Arteries

PLATE 7

FIG. 5. Thickened blood vessel wall with connective tissue, either endarteritis or an organized thrombus. × 210.

FIG. 6. Connective tissue narrowing the lumen of an artery with cells suggesting smooth muscle cells, presumably an old organized mural thrombus. × 210.



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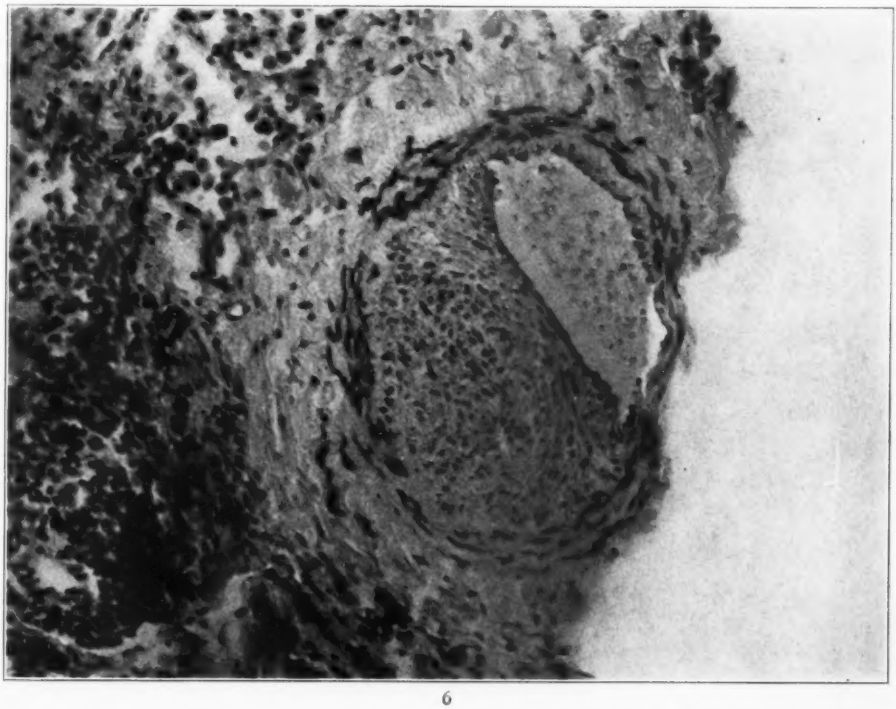

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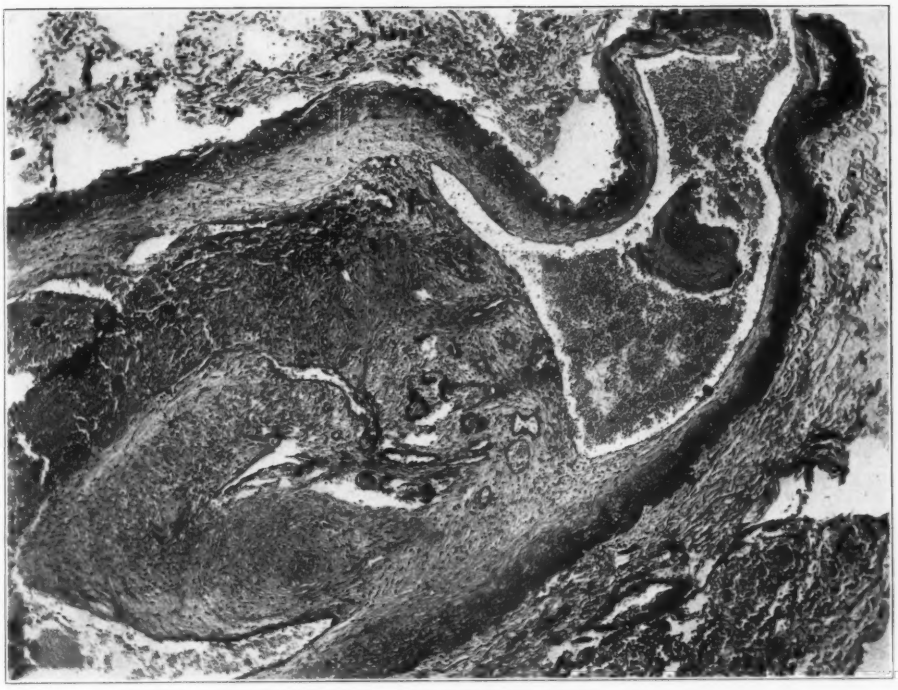
Frothingham Thrombosis of Branches of Pulmonary Arteries

PLATE 8

FIG. 7. Thrombus in an artery with canalization in the thrombus. × 62.

FIG. 8. Fresh thrombus with early organization in an artery. Arterial wall in good condition. × 62.



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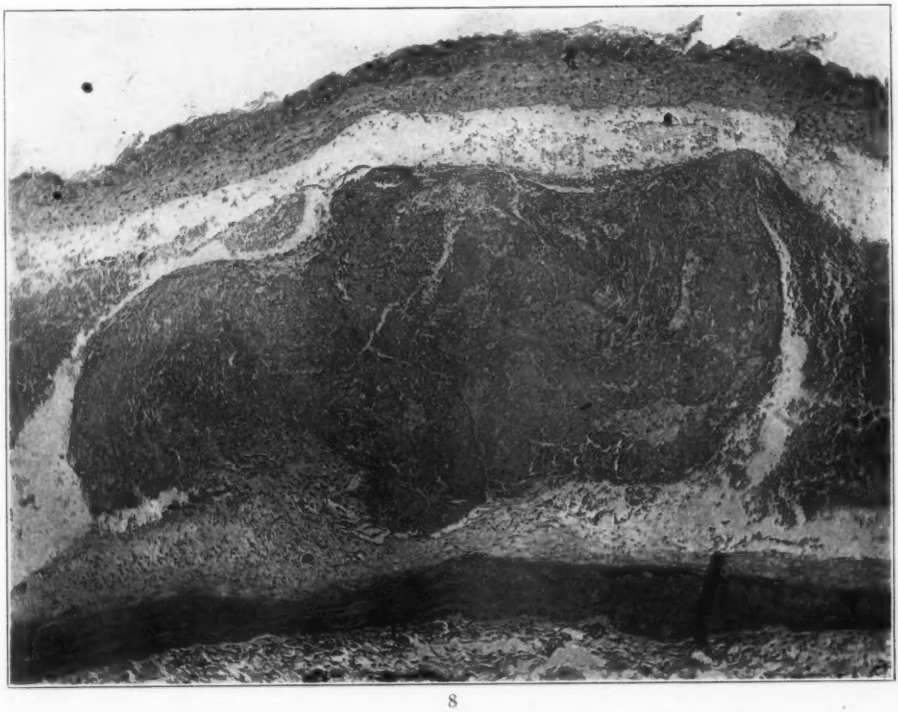

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Frothingham Thrombosis of Branches of Pulmonary Arteries

A CASE OF MAMMARY GLAND TISSUE IN THE AXILLA *

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In the monograph upon "The Breast: Its Anomalies, Its Diseases and Their Treatment" by Deaver and McFarland,[1] some ninety-five publications recording more than a hundred cases of axillary mammary tissue are reviewed, and arranged as follows:

I. Cases in which lumps of painful character appear in the axilla during pregnancy or at the beginning of lactation, either where small painless lumps have existed since puberty, or where the patient was unconscious of the existence of any abnormality.
II. Cases in which "axillary lumps," similar to those mentioned, occur under like conditions, but discharge a milky secretion through openings of varying size and number.
III. Cases in which the "axillary lumps" are surmounted by a more or less distinct areola with one or more rudimentary nipples.
IV. Cases in which there is a distinct mammary gland in the axilla, upon which there is a distinct areola and a fairly well formed nipple.

The following case seems to belong in Group I of this tabulation, that in which the greatest number of incidences occur.

REPORT OF CASE

The patient is a young married woman, 23 years of age, who two years ago gave birth to her first child. At the usual time after delivery the breasts swelled and a lump occurred in the right axilla that quickly grew to the size of a hen's egg.

A binder was applied to the breasts, which diminished in size, and at the same time the lump in the axilla grew smaller although it never went away.

Not long ago the patient spoke of having a "lump under her arm," and her brother-in-law, who is a surgeon, became interested and suggested an examination at which another similar but smaller "lump" was found in the other axilla. The surgeon now became apprehensive, fearing that they were enlarged lymph nodes, and possibly indicative of beginning disease of the lymphatic system.

* Received for publication June 14, 1928.

The lump in the right axilla caused a visible swelling when the arm was extended, and when carefully palpated seemed to be about 4 by 2 cm. in size and resolved itself into three rounded lobules or portions, one free and two connected like a dumb-bell. All were insensitive and movable, except for intimate connection with the skin. The patient consenting, the larger "lump" was removed under local anesthetic and no enlarged lymph nodes were found. Incision of the excised tissue resulted in the escape of whitish fluid that looked like milk.

Microscopic examination revealed a histological structure corresponding to that of the mammary gland.

This led to further interrogation of the patient, from whom it was learned that the lumps in the axillae enlarged and became a little tender at each monthly period but that they never "leaked" or discharged any milky fluid either at the time of the pregnancy enlargement that led to their first observation, or at any time since.

Resemblance between the large sudoriparous glands of the axilla and mammary tissue have sometimes caused the former to be mistaken for the latter. The coiled tubules of the eccrine glands do resemble the smaller lobules, and the broad lumenated apocrine glands suggest the "residual secretory acini" of the mamma.

Inasmuch as both the sudoriparous and lactiferous glands are so closely related in structure that the latter is looked upon as an exaggerated and specialized homologue of the former, there is every reason that the histological resemblances should be close.

Each sudoriparous gland seems to consist of a single tubule (it is said that in some of the larger glands the tubule may give off a branch), while a mammary gland is composed of a highly complex system of branching tubules. In each there is a secretory and a conducting portion whose structure varies with its function. The secretory portion of each tubule of each gland has the same theoretical fundamental structure, and consists of a membrana propria surmounted by two layers of cells, the outer and less conspicuous sometimes known as the "basket cells" being regarded as ectodermal involuntary muscle cells, the inner much larger and cuboidal, known as the "chief" or secretory cells. The prominence of the latter as compared with the former, sometimes gives the false impression of tubules lined by a single layer of cells. Again, and especially in the mammary glands, the contraction of the muscle cells seems to cause

crowding of the secretory cells, to disguise their relation to the basement membrane and to confuse the cells of the two layers so as to give the impression of several layers of cells with no limiting membrane. As the ever increasing quantity of secretion requires larger and larger tubules to accommodate it, the tubules of the lactiferous glands unite with others, and still others, as they approach the nipple at which their common outlets are situated, eventuating in large ducts lined with columnar epithelium, and terminating in ampullae.

The tubules of the sudoriparous glands receiving no accessions, remain approximately of uniform diameter except that during active secretion they dilate widely. But as the secreting part of the tubule ends and the conducting part is reached, instead of expanding as is the case with the lactiferous glands, it diminishes to about one-half its former diameter, loses its muscle cells, and develops a double or treble cell layer with a homogeneous zone or cuticle about the lumen.

The larger apocrine glands are less compact, the lumens are wider, the epithelium more flattened, and the cytoplasm of the cells distinctly eosinophilic.

But apart from these simple histological considerations, there is a remarkable difference in behavior that is based upon the respective functions of the different glands.

The sudoriparous glands although not constantly active, must ever be ready to assume their important functions, and, therefore, have a structure that may be looked upon as permanent and invariable, while the mammary glands are only occasionally called upon to exercise their function, for which there must be elaborate preparation. Their structure, therefore, varies from time to time to an extraordinary degree, accordingly as they are resting, or in process of evolution or involution.

The outset of estruation in animals, and menstruation in humans, is marked by phenomena that vary in extent in different animals, and in degree in different individuals. It is now pretty well agreed that these are the result of the exciting effects of a hormone originating in the ovary and probably derived from the liquor folliculi or secreted by the corpora lutea.[2]

There is at present no suspicion that this hormone in any manner affects the histology of the sudoriparous glands, but its effect upon the lactiferous glands is striking, for their previously quiescent

tubules begin to extend and branch until distinct lobules of mammary tissue are formed.

As it is at estruation that the sexes usually unite and, therefore, at that time that conception takes place, it might not be wide of the mark to conclude that this growth of mammary tissue is in anticipation of expected gravidity, and to gain time for the voluminous increase of tissue that must nourish the resulting offspring. If no conception follows, the termination of estruation is followed by atrophy of the useless glandular tissue, and the return of the gland to its "resting stage."

The application of this knowledge to what takes place in the axillae of those rare cases in which women have lactiferous gland tissue in that situation, is sufficient to explain the clinical phenomena. During virginity they may never suspect such an anomaly, but when pregnancy occurs, the gradual growth of all lactiferous tissue may result in the appearance of a "lump" wherever such tissue is situated, and when lactation sets in, any such "lump" may undergo a sudden and painful increase because of the secretion and retention of the milk. If the milk escapes or can be expressed the pain is relieved; if that cannot be done further painful enlargement may occur and become so severe as to necessitate operation. In ordinary cases the pain soon subsides and the lump gradually diminishes, sometimes disappearing altogether. But in many cases the patient can find it by palpation, and finds it sometimes sensitive at the menstrual periods, because of the slight hypertrophy of the lactiferous tissues at that time.

In the present case knowledge of the histological cyclical variations in lactiferous tissue may be of assistance in distinguishing between the sudoriparous and lactiferous tissues where they come into close juxtaposition, as is so often the case in the axilla. The biopsy, at which the tissue obtained for microscopic examination was secured from the patient, was performed on the first day of a menstrual period and only one hour before the flow began. At that period each unit of the lactiferous tissue should be surrounded by a loose zone of periductal tissue, and should be giving off those auxillary ductules or acini that form the periodically appearing lobules.

As a matter of fact, precisely that condition obtains. There is proliferation of the glandular tissue, and some secretion fills the ducts of the mammary tissue.

It seems, therefore, that the clinical history of the case, the surgical discovery of a collection of milk at the time of the operative removal of the tissue, and the histological findings all point clearly to this case as one of mammary tissue in the axilla.

REFERENCES

1. Deaver, J. B., and McFarland, J. The Breast: Its Anomalies, Its Diseases and their Treatment. P. Blakiston's Son & Co., Philadelphia, 1917, 55.

2. Rosenberg, A. *Frankfurt. Ztschr. f. Path.*, 1922, xxvii, 466.

DESCRIPTION OF PLATE

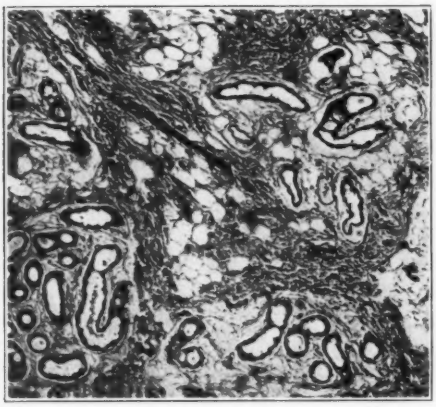
PLATE 9

FIG. 1. An eccrine gland below on the left, apocrine glands elsewhere. This is from the superficial part of the removed tissue.

FIG. 2. Below and to the left three mammary lobules, two partly blended, the third separate. Elsewhere apocrine tubes of sudoriparous glands.

FIG. 3. More deeply in the removed tissue one finds mammary tissue only.

FIG. 4. Various lobules of mammary tissue in the deeper part of the removed tissue.



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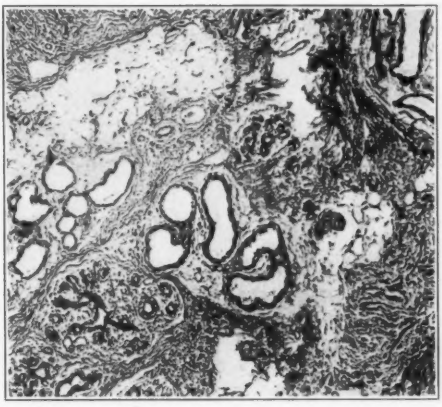

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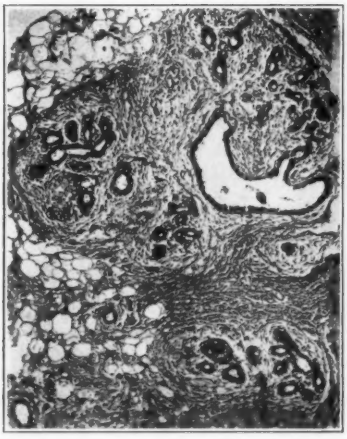

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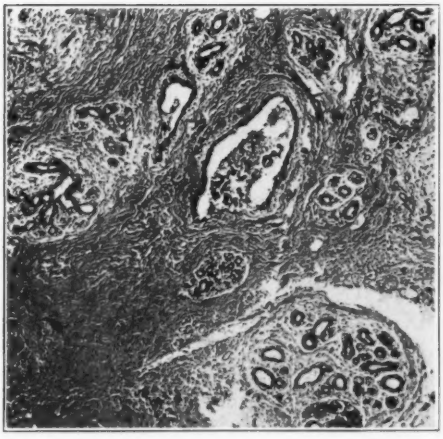

4

McFarland

Mammary Gland Tissue in Axilla

MALIGNANT THYMOMA WITH METASTASES *

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The case of malignant thymoma reported here presents clinical and pathological features of unusual interest and our observations are recorded with the hope of contributing information that may aid in arriving at a correct diagnosis in an early stage of a condition heretofore recognized only in the advanced form and then only after biopsy and in the majority of cases only after autopsy.

Throughout the early months of our case, we met many diagnostic difficulties which were augmented rather than lessened by recourse to laboratory assistance and it is believed that an accurate presentation of our observations will tend to a better understanding of the problems met in thymic neoplasms of a malignant nature.

The term thymoma is misleading because it simply implies a tumor, benign or malignant originating in the thymus gland; however, since that appellation was first proposed by Ambrosini [1] in 1894 it has been generally adopted to describe an atypical malignant neoplasm of the thymus gland.

The pathology of a thymoma is still a matter of controversy and will remain so until histologists agree upon the normal constituents of the thymic parenchyma. A satisfactory discussion of this subject entails a review of the embryology, histogenesis and pathology of the thymus gland and as that has been presented in recent writings by Foot,[2] Jacobson,[3] and Symmers and Vance,[4] it seems superfluous to enter such a discussion here. We will, however, refer to these articles where points relevant to our case are encountered.

In describing the disease entity called thymoma we quote Ewing's [5] description, not only because he is a recognized authority on the subject but also because he was a consultant in our case. According to him a thymoma is a lymphosarcoma of the thymus gland, composed of thymic or reticular cells, occupying the anterior mediastinum extending from the sternal notch or as high as the thyroid gland down to the diaphragm. A thymoma usually surrounds

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and compresses the trachea, bronchi, pericardium and great vessels. By compression and occasionally by invasion of vessels and air passages death is caused by asphyxia and venous obstruction which may be gradual or sudden. The tumor mass is encapsulated within the mediastinum in some cases but in others it becomes adherent to surrounding organs and invades the pleura, lung, pericardium, walls or lumina of vessels, trachea, bronchial, cervical and axillary lymph nodes. Metastases to distant organs are occasionally noted involving spleen, liver, adrenals, pancreas, kidney and bone marrow of the humerus (Zniniewicz).[6] In our case there were also metastases to the stomach and to one ovary. Strauss[7] reports a case in which the tumor invaded the musculature of the auricles and completely obliterated the lumen of the vena cava by pressure. The pericardium was involved in our case but the myocardium escaped invasion.

Thymoma is an uncommon neoplasm and only about ninety cases have been reported, which include those in which neither biopsy nor autopsy were done and hence the accuracy of the diagnosis may be questioned. The sixty-nine cases of tumor of thymic origin which Rubaschow[8] collected from the literature include both sarcomas and carcinomas and it is doubtful that all these cases would be classed as thymomas in the light of more modern research. Numerous cases have been reported with distant metastases yet only three (Ambrosini,[1] Symmers and Vance,[4] Friedlander and Foot[9]) are on record in which the process invaded organs below the diaphragm and ours makes the fourth.

Although the literature on thymoma is quite extensive but little reference is made to its clinical phases, most of the discussions being confined to the pathological findings. In searching the literature it was hoped we would receive some enlightenment from the clinical experiences of others that might aid in making an early diagnosis but in the majority of cases heretofore reported no statement is made whether the correct diagnosis was arrived at before resorting to biopsy. We note, however, that some observers met with diagnostic experiences similar to ours and it is of interest to compare them. Foot and Harrington[10] reported a case of thymoma in which the original diagnosis was unresolved pneumonia. Miller[11] records a patient whose diagnosis lay between a laryngeal diphtheria, heart disease, a foreign body in the air passages, asthma and some vague

intrathoracic growth. Jacobson [3] in reporting a case of primary neoplasm of the thymus emphasizes the difficulty of an ante mortem diagnosis. His case was originally diagnosed muscular rheumatism by a local physician and later a surgeon considered the condition one of metastatic cancer of the spine. Apparently the early manifestations directed attention to the metastatic condition rather than the primary focus. Even the roentgen-ray examinations in this case were misleading for they pictured a process of the spine simulating Pott's disease and metastatic infiltration of the lungs. To cite another example of the difficulty of an early diagnosis we mention the experience of Helvestine [12] who reports a case in which the first diagnosis was intrathoracic tumor with tumor of the neck, possibly a combination of Hodgkin's disease with exophthalmic goiter. Our case presented similar diagnostic difficulties and only after repeated biopsies and pathological studies was a correct diagnosis finally made. In the case we report below, the diagnoses varied with the progress of the disease and were more or less influenced by certain laboratory studies and roentgenograms. At various stages the clinical signs were those of non-toxic substernal thyroid, status thymicolymphaticus, lymphosarcoma, and Hodgkin's disease. The two former diagnoses were at first confirmed by roentgen rays, but serological and histological examinations misled us in suspecting syphilis and later tuberculosis. Subsequent X-rays however disclosed a distinct mediastinal newgrowth and the final biopsy presented the histological picture of thymoma.

Report of Case

Clinical Report: A. H., a white female of the phthisical status, aged 25 years. First seen July 27, 1927.

Chief Complaint: Swelling of neck of three weeks duration interfering slightly with breathing; some pain at base of neck.

Family History: Mother died of cancer of the stomach.

Past History: Tonsillectomy in childhood.

Present History: Noticed swelling in thyroid region of neck about three weeks ago accompanied by no other symptoms at that time, except an "embarrassing" pinkish discoloration of eyelids and brows. More recently she complained of becoming easily fatigued on exertion and feels somewhat nervous. The thyroid swelling is slowly but steadily increasing. There is a tendency to shortness of breath.

Physical Examination: Well developed, fairly well nourished female. Pale complexion with an unusual sharply circumscribed pinkish discoloration involving eyelids and brows, giving the impression of a theatrical "make up."

Eyes: Reacted normally to light and accommodation and were equal on both sides.

Nose and Teeth: Negative.

Throat: Ulcer in vault of pharynx. Tonsils, removed.

Thyroid: Uniformly enlarged but of a greater density on palpation than is usual in simple goiter or in Graves' disease. There were no nodular masses as in adenoma of the thyroid or in tuberculous glands or Hodgkin's disease.

Chest: No deformities; no pulsation; no bulging; no engorgement of veins of chest or arms. Substernal dullness 9 cm. in diameter extending from the thyroidal enlargement down to the base of the heart.

Heart: Showed no abnormality excepting that the apex measured only 7 cm. from the midsternal line. No arrhythmia, no murmurs, no thrills; a normal rate of 72 to the minute.

Lungs: Negative except for the above-mentioned dullness.

Abdomen: Liver and spleen not palpable and no other signs of abnormality.

Extremities: Negative excepting for eczema of elbows. No clubbing of fingers.

Blood Pressure: Systolic 120; Diastolic 80. No difference between right and left arm.

Superficial Reflexes: Normal.

Superficial Lymph Nodes: Not palpable. *Peripheral vessels* not thickened.

Tentative Diagnosis: Substernal, non-toxic goiter.

Laboratory Findings: To this date were essentially negative.

Progress Notes

August 11, 1927: Slight swelling of cervical glands was noted. Wassermann taken and reported negative.

August 18: Admitted to hospital for further study. Physical examination confirmed above findings except for an increased heart rate of 96 per minute with frequent premature contractions. The sounds were of good quality without murmurs. *Lungs* and *abdomen* presented no changes since previous examination.

August 20: A roentgenogram at this time showed a thorax long in its vertical diameter with the heart small and inclined to the rotate type. An increase in density in the superior mediastinal region is noted especially on the right side together with a clouding above this suggesting probable substernal thyroid.

August 24: Basal metabolism reported normal. Further study of the roentgenogram led to the belief that the substernal shadow was attributable to a persistent thymus rather than to a substernal thyroid, which fact, together with a small heart and the general lymphatic type of individual, justified the revised diagnosis of "status thymicolymphaticus."

A *blood count* done at this time gave the following figures: Reds, 5,090,000; whites 7,900; hemoglobin 87 per cent.

Differential Count: Polymorphonuclears 69 per cent; transitionals, 1 per cent; small lymphocytes 20 per cent; large lymphocytes 5 per cent.

Temperature readings varied from 99° F to 100° F.

Weight: Remained about stationary at 107 lbs. The patient was treated symptomatically and kept under observation. The shortness of breath, insomnia and general malaise continued to increase and the cervical glands gradually grew larger.

September 9: Biopsy was done on gland removed from the cervical region and the patient discharged from the hospital. Following are the results of this biopsy.

Microscopic Examination

Sections show several small lymph glands embedded in dense fibrous tissue. The lymph glands are circumscribed in part and in part the outlines are diffuse. Within the lymphoid substance there are no well demarkated follicles, but rather a picture of diffuse lymphocytosis. Scattered among the lymphocytes are numerous plasma cells and occasional eosinophilic polymorphonuclear leucocytes. The poorly demarcated lymph glands show numerous lymphocytes, plasma cells and eosinophiles infiltrating in all directions throughout the surrounding fibrous tissue. The fibrous tissue extends into the attached fatty tissue with infiltration by lymphocytes, plasma cells and eosinophilic leucocytes. There is a large necrotic area, chiefly in the fibrous tissue. In this necrotic area remnants of cell nuclei can be seen and occasional blood vessels and some necrotic portions of lymphoid tissue.

The striking thing in the fibrous tissue is the thickening of the walls of many blood vessels with perivascular infiltration by lymphocytes and plasma cells. The presence of numerous plasma cells and eosinophilic leucocytes together with the fibrous tissue proliferation and especially the type of necrosis, suggest a syphilitic lesion.

Diagnosis: Chronic lymphadenitis and perilymphadenitis with necrosis suggesting syphilis. In the absence of definite evidence of a newgrowth, it seems more than likely that syphilis is the proper diagnosis. We would suggest a further Wassermann test be made, preferably on the spinal fluid.

Pursuant to the suggestion of the pathologist another blood Wassermann test was made which was again negative. A similar test on the spinal fluid was not obtained. In view of the conflicting information thus obtained it was deemed advisable to send a biopsy specimen to another pathologist and Dr. Ewing was consulted. His expressed opinion follows: "The picture *resembles* tuberculosis but must be *interpreted* as a distant effect produced by radiation employed in the thymic region." For example, it is common, according to Dr. Ewing, for axillary lymph nodes to show a picture simulating tuberculosis when radiation has been applied to cancer of the breast. Accordingly a revised pathological diagnosis was rendered of lymphadeniasis with necrosis, produced by the distant effect of radiation.

Previous to receiving this report we had clinically excluded tuberculosis which conclusion had been subsequently confirmed by the X-ray findings.

September 21, 1927: A provocative salvarsan was followed by a negative Wassermann.

September 25: The substernal mass subjected to one X-ray treatment.

September 26: Vernes' flocculation test for syphilis was negative.

September 28: Patient was again admitted to the hospital for further observation and for X-ray therapy. The only complaint now being stiffness and a sense of soreness in the neck upon arising in the mornings. There had occurred a loss of 3 lbs. in weight during the last six weeks. The temperature continued to hover around 99° F and 100° F and it was deemed advisable to postpone further X-ray therapeutics until the fever subsided.

October 5: A Vernes' flocculation test for tuberculosis gave a report of 80 and 83 milligrams of flocculation per 1 cc. of fluid with a note from the technician stating that such high results denoted active tuberculosis. This observation in conjunction with the pathologist's previous diagnosis of tuberculosis after studying the first biopsy specimen together with the developing lung involvement led us to suspect an acid-fast infection. Repeated sputum examinations however failed to discover the presence of tubercle bacilli.

October 8: An annoying cough with some pain in right chest developed. Examination of the chest revealed dullness at the base of the right lung posteriorly with feeble yet audible breath sounds and a diagnosis of pleurisy was made. A roentgenogram reported a marked reduction in the size of the tumefaction in the retrosternal space though tumefaction extending downwards from the cervical region is still noted. The picture however has been altered by the addition of a low-grade bronchopneumonic or bronchiectatic infiltration involving the right lower lobe. There is also pleuritic involvement with slight exudate, probably plastic in type obscuring the right costophrenic sinus. The heart is of the mitral stenotic type, relatively small in its total area with a marked straightening of the left heart border. The roentgenological diagnosis being, "probable thyrcidal enlargement; reductien in size of substernal mass; low-grade bronchopneumonic process lower right lung with pleural involvement at right base."

October 14: The dyspnea now caused decided distress and the clinical signs of pleural effusion were unmistakable.

Roentgenological examination was reported as follows: Examination of the thorax shows marked mediastinal infiltration with a low-grade bronchopneumonic process pushing into the left central lung field as well as toward the right base. On the right side the lung condition is partly obscured by concomitant pleural thickening, both interlobar and basal, along with a considerable amount of exudate obscuring the lower half of the right pulmonic field. Evidence of pleural involvement is noted in the left costophrenic sinus.

Diagnosis: Marked mediastinitis, central low-grade bronchopneumonic infiltration. Thoracentesis was done and 1320 cc. of a slightly hazy straw-colored fluid was removed and sent to the laboratory for differential cell count, for presence of mitotic division figures and for inoculation in guinea pig. The report is as follows: "Sediment contains numerous mononuclears, moderate poly-

morphonuclear leucocytes and lymphocytes. No tumor cells recognized. No mitotic division figures found. Color, orange; translucence, moderate; consistency, fluid; butyric acid, plus four; white cells per c. mm. 1270; polymorphonuclear leucocytes 11 per cent; lymphocytes 89 per cent." All subsequent reports on the results of guinea pig inoculation were negative for tuberculosis.

A consultation with an eminent clinician was held at this time who made a diagnosis of Hodgkin's disease. It may be stated here that those of us who were most intimately connected with this case had at the very beginning considered the possibility of Hodgkin's disease but had ruled it out for want of clinical evidence, a finding which was subsequently confirmed by study of the tissue removed at the first biopsy. However in deference to this opinion, and because we were not fully convinced that either the clinical or pathological diagnoses were correct another biopsy was ordered. Because of conflicting interpretations in previous pathological studies we sent tissue from this biopsy to several laboratories where different pathologists rendered the following opinions.

First Laboratory Report: "Microscopic sections show no recognizable lymph gland structure. We find instead a diffuse growth of reticular cells with small lymphocytes scattered throughout but not in great numbers. The reticular cells vary from round to polyhedral in shape and vary markedly in size, many of them being very large, and the general picture is that of homogeneous sheets of cells. It would seem that the lymphocytes play a passive rôle in the formation of the tumor. It is usual in this particular group of tumors that when the reticular cells become so numerous the lymphocytes largely disappear. The neoplastic activity is not circumscribed but extends outward into the surrounding fibrous tissue and fat. Mitotic figures are occasionally found but not in all oil immersion fields.

Diagnosis: Thymoma. (Fig. 1.)

A consultation was held over this study with Dr. Ewing who concurred in the diagnosis made by our pathologist. His decision was accepted as final.

Second Laboratory Report: Microscopic sections show the normal architecture of the lymph node replaced by a diffuse lymphoid cell infiltration with some increase in the fibrous tissue comprising the stroma. Scattered eosinophiles are also found in the tissue. Histologically the tissue indicates a chronic (granulomatous) inflammation, not induced by the tubercle bacillus and probably not luetic. The presence of eosinophiles without the special cells characteristic of Hodgkin's disease precludes fairly definitely the possibility of this disease.

Diagnosis: Chronic (granulomatous) inflammation.

October 21, 1927: Patient is still running a sustained temperature of 100° to 102° F with little change in the swelling of the neck and cough or in her general condition.

October 22: Following the last biopsy report it was decided to treat the condition with radium. Accordingly 10 radium seeds were implanted in the mass in the left cervical region.

October 25: Patient was transferred at the request of the family to a sanitarium where she died on November 20, 1927.

Report of Autopsy

Body: Is that of an extremely emaciated white female aged 24, 163 cm. long. Abdomen scaphoid. General rigor and dependent livor mortis. Skin everywhere is covered with numerous discrete dry eruptions, 1 to 2 mm. wide, similar in appearance to old dried small scratch marks, the skin immediately adjacent appearing normal. On the mucocutaneous junction of left lower lip there is an irregular excoriation, 10 by 12 mm. Three old vertical linear scars are present in the left neck, 3, 3 and 2 cm. long. There is a small, dry, shallow decubitus ulcer over the left sacrum. In lower abdomen, just above both Poupart's ligaments and parallel to them are bluish ecchymotic areas, those on the right being more marked and larger, measuring 11.5 by 4 cm. Both supra- and infraclavicular fossae and axillary spaces contain numerous hard, discrete nodules, the largest being about the size of lima beans. In the subcutaneous tissues of left lateral thoracic wall in anterior axillary line is another indurated, freely movable nodule, 1.5 cm. in diameter. Just to the right of the sternum in the second intercostal space is a smooth, hard elevation, measuring 4.5 by 3.5 cm.

Both breasts are small, soft, and contain indefinite masses of soft mammary tissue. Pupils are regular, equal, moderately dilated, 6 mm. in diameter. There is a bilateral pterygium on nasal side of each eye. Teeth are good. Mucous membrane pale.

Incision: Y-shaped from midsternum to symphysis pubis. Panniculus adiposis practically absent. Muscles flabby and pale red.

Neck: Numerous hard superficial and deep cervical lymph nodes are present, the largest measuring 2 by 1 by 1 cm. In the anterior plane just beneath the very thin dark yellow subcutaneous fat is a very firm, fibrous opaquely white mass, 6 by 3 by 1.5 cm., with a tongue-like lower border fitting into the suprasternal notch. Thyroid is compressed into a thin colloid-containing mass. A lateral lobe measures 2.5 by 1.2 cm.

Thorax: (Fig. 2.) Left pleural cavity is almost completely obliterated, except for several locculi, about 5 cm. in diameter, containing turbid dark yellow fluid. In right pleural cavity there are about 500 cc. of blood-tinged fluid and a few firm fibrous adhesions posteriorly. Both parietal and visceral pleurae of the lateral wall and diaphragmatic surfaces are covered with a dirty gray fibrinous exudate. Visceral pleura of lower right lobe, especially the diaphragmatic portion, is greatly thickened and pearly white (Fig. 3). Parenchyma of right and left lungs are dark red and very edematous. Upper right lobe posteriorly, near the hilum, contains a metastatic nodule 2 by 1.5 cm. in diameter, hard, very cellular. Anteriorly there is another, beneath pleura, 5 mm. in diameter. In left hilum are several similar nodules between the bronchial ramifications. Left apex is infiltrated with cellular, opaquely gray tumor tissue. There is a protuberant indurated growth, left paramedian, on the upper surface of the diaphragm, 5 by 3 cm., cut surface showing fibrous and more grayish cellular areas.

The external nodule in the right second interspace corresponds to a similar growth of equal size interiorly (Fig. 2) and merges with the mediastinal mass which pushes the heart over to the left. Pericardial sac contains about 150 cc. of blood-tinged fluid. Whole sac (Fig. 4) is lined by a shaggy, villous, brownish, fibrinous membrane, easily pulled off. Sac after formalin fixation measures 10 by 10 by 12 cm. *Heart* is in systole, 235 gm., 8.2 by 10 by 5 cm. All the coronary arteries are covered with tumor growth, the posterior branch measuring 9 mm. in its outer diameter. Aorta shows numerous small yellow raised atheromatous patches. Right ventricular wall, 0.5 cm., left 2 cm. Mitral valve shows many old vegetations. Pericardium in region of coronaries is as much as 5 mm. thick, firm and opaquely white.

Anterior mediastinal space is completely replaced by tumor tissue from superior border of sternum down to the diaphragm, to a depth of 4 cm. Superior thoracic aperture is completely infiltrated with opaquely white, hard tumor, completely surrounding trachea and esophagus. In fact the whole mediastinum down to esophagus in the midthoracic region consists of this firm opaque tumor tissue.

Abdomen: No free fluid present in peritoneal cavity. Large intestines are but slightly distended; several pultaceous small fecal masses are present in the terminal ileum. Stomach contains about

400 cc. of green, sour smelling fluid and some air. Gastric mucosa throughout shows quite a number of split-pea-sized grayish elevations, the apices of which contain a shallow ulcer about 3 mm. in diameter. Many perigastric glands are enlarged, resilient, the largest measuring 3 by 1.7 by 1.5 cm. Mesentery of ileum shows many minute to pea-sized calcified nodes. Intestinal mucosa is deeply injected and covered with blood-tinged mucus.

Liver: Measures 17 by 22 by 8 cm., firm and yellowish brown in color. No metastatic infiltration is present grossly on section. *Gallbladder* measures 5 by 2 cm., wall thin. Contents, about 5 cc. of dark green bile. *Spleen*, 8 by 5.5 by 2.5 cm., is soft, dark red and very pulpy. No tumor nodules found. *Pancreas* is firm and grayish yellow.

Left kidney, 285 gm., 13 by 8 by 6 cm.; *right* 275 gm., 13.5 by 7.5 by 5.5 cm. Their surfaces are coarsely lobulated. Capsule strips easily, exposing a mottled grayish red, smooth, swollen surface (Fig. 5). On section a similar mottled variegated dark red and opaquely gray appearance is noted. The medulla, which grossly seems to be uninvolved by the tumor infiltration appears as streaks of dark red radially towards the renal papilla. *Ureters* are grossly negative; the right passes from the left side, paramedian, over the bodies of the vertebrae towards the right brim of the pelvis. Right kidney here is obliquely situated, its lower pole lying in the left abdomen. *Bladder* is contracted, containing a few centimeters of slightly turbid urine. Mucosa is pale and very much rugose. *Adrenals* show no gross findings.

Uterus: Measures 6 by 5.5 by 2.5 cm., wall at fundus being 1.2 cm. thick. Mucosa is thin and pale. *Right ovary*, 4.5 by 2 by 1.5 cm., and left 5 by 2 by 1 cm., containing corpora lutea and albicantes. Tubal ostia are patent. Serosa of uterus and adnexae are deep purplish red in color.

Anatomical Diagnoses

Thymoma with extensions and metastases to lungs, peribronchial, cervical and axillary lymph nodes, stomach, kidneys and retroperitoneal and gastric lymph nodes. Acute fibrinous pericarditis (cor villosum) with effusion. Edema and congestion of lungs; chronic adhesive pleuritis (bilateral); hydrothorax (bilateral); acute fibrinous pleuritis (right). Healed tuberculosis of the mesenteric lymph nodes.

Microscopic Findings

Neck: Lymph nodes from neck show a diffuse necrosis, appearing as shadows of previous cells which refuse to stain differentially. However, there still remain nests of viable-looking tumor cells of the reticulum cell type of thymoma (Fig. 1), large polyhedral cells with large vesicular nuclei, distinct nucleoli and acidophile cytoplasm. Mitotic figures are numerous. The general picture of thrombosis, necrosis and fibrosis may be interpreted as a radiation effect on the tissues.

Mediastinum: Sections from the main tumor mass in the anterior mediastinum, pulmonary nodules, diaphragmatic and enlarged axillary nodes all present a similar picture of diffuse tumor cell overgrowth, made up of large polygonal cells with vesicular nuclei, distinct nucleoli and acidophile cytoplasm. Numerous mitotic figures may be seen. Similar tumor cells invade the peri-esophageal and peritracheal tissues. Vessels here contain fibrin thrombi. Alveoli of lungs near area of tumor infiltration contain dense fibrinous exudate, coagulated serum or red blood cells. Vessels in this region are engorged with blood or plugged with thrombi. Areas of necrosis are present. Beneath the fibrinous exudate the subpleural tissues are infiltrated with tumor cells.

Heart: Beneath the dense fibrinous exudate on the visceral and parietal pericardium is present a similar tumor infiltration as seen under the pleura (Fig. 6). Myocardium itself is not involved.

Gastro-Intestinal Tract: Gastric and intestinal mucosae show marked postmortem decomposition, are injected and infiltrated with numerous round cells. The lymphocytes of a large perigastric lymph node are completely replaced by tumor elements. Submucosal vessels are deeply engorged. In sections from the nodules of the gastric mucosa tumor cells and mitotic figures are plentiful (Fig. 7).

Liver, Pancreas and Spleen: Essentially negative.

Kidneys and Adrenals: A widespread tumor replacement of renal tissue leaves very little cortical parenchyma here and there (Fig. 8). In the more involved areas the glomeruli can be seen as single entities, lying in a bed of congested tumor tissue. Adrenals are negative except for minute focal petechiae in both cortex and medulla.

Uterus and Adnexae: Show a general congestion. One section of the ovary shows metastatic tumor areas with many mitotic figures.

Final Diagnoses

Thymoma with extension to pleura, lungs, pericardium, diaphragm, and metastases to cervical, peribronchial and axillary lymph nodes, stomach, perigastric lymph nodes, kidneys, ovary, and invasion of sternum. Acute fibrinous pleurisy associated with tumor cells. Hydrothorax. Acute fibrinous pericarditis with effusion associated with tumor cells. Edema and congestion of lungs. Chronic adhesive pleuritis (bilateral). Healed tuberculosis of mesenteric lymph nodes. Chronic passive congestion of liver and spleen.

Comment

This case of malignant thymoma presents many points of clinical and pathological interest, the outstanding features of which are: (*a*) the difficulties in reaching a definite conclusion as to the true nature of the process involved; (*b*) the apparently slow growth of the neoplasm during the early months of progress; (*c*) the absence of any but relatively trivial clinical symptoms; and (*d*) the extensive metastases to distant regions without definite disturbances in function of the organs involved.

Attention has already been called to the confusion caused by early pathological, serological and roentgenological studies. The biopsy findings show the importance of obtaining a biopsy before the therapeutic use of the roentgen ray. Our reliance upon the results of the Vernes' flocculation test for tuberculosis has received some justification in a recent contribution by Baylis,[13] whose observations in a series of 250 cases explains our error in interpreting the figures recorded. It appears that positive results are obtained in the presence of pulmonary newgrowths of non-tubercular origin which have undergone necrosis, and are therefore unreliable as an indication of active tuberculosis. Clinically the latter condition was ruled out at the first exmination, for there were no physical signs and no subjective complaints suggestive of a Koch infection. The substernal and subclavicular dullness was continuous with the mass in the neck and was not attributed to pulmonary consolidation. At this stage of the disease there were no sharply outlined enlarged glands as seen in tuberculosis; on the contrary the left cervical mass consisted of a single diffuse, goiter-like tumor. It was not until a microscopical examination of gland tissue showed changes suggestive of tuber-

culosis together with a positive Vernes' test with the subsequent development of pleurisy, that our attention was again attracted to the possibility of a tubercular process.

A diagnosis of syphilis was entertained because of the persistent ulceration of the pharynx of unknown origin which resisted ordinary medication. Repeated Wassermanns however were negative. Later when a histological study of the tissue removed at biopsy suggested a syphilitic lesion, a provocative Wassermann and a Vernes' flocculation test for lues were done, but again reported negative. Lymphosarcoma and Hodgkin's disease had been given due consideration but the appearance of the neck was not typical of these conditions and the first biopsy showed none of the characteristic histological changes ascribable to them.

The apparently slow growth during the early stages of our case followed by a rapid progress in the last two months is in accordance with the description of similar cases of thymoma reported by other observers. Apparently the process grew so insidiously that the structures of the thorax gradually accommodated themselves to the invading mass without producing marked pressure symptoms. Considering the size of the neoplasm removed at autopsy it is astonishing that the patient presented so few symptoms during life. The duration of our case from the time when the patient first consulted us until death was a little short of four months, during the first two months of that period the patient's chief concern was the enlargement of the neck and the disfiguring discoloration surrounding the eyes. She complained of a slight cough, soreness of the neck, dysphagia, nervousness, insomnia and loss of appetite, but all these disturbances disappeared on occasions and she would then enjoy intervals of perfect comfort. It was surprising how well she felt considering the seriousness of her condition and we were impressed with the patient's cheerfulness and state of optimism, a mental attitude comparable to that so often attributed to subjects of tuberculosis. Not until the lung parenchyma became invaded, about seven weeks prior to death, did she suffer real discomfort. She then complained of an annoying cough with considerable pain in the right chest. It was also surprising how little disturbance was occasioned by the metastases to distant organs. The kidney parenchyma showed widespread replacement by tumor elements yet the last urinalysis was negative except that only 400 cc. were excreted during twenty-four

hours. Unfortunately a blood chemistry was not done to determine whether nitrogen retention was present.

Attention is also directed to the manner of invasion of the lungs. Previous observers reported the involvement of lung tissue by direct extension whereas in our case this was followed both by metastasis and extension. The extension over the visceral and parietal pericardium was by continuity from the primary tumor mass. The peculiar pinkish color phenomenon surrounding the eyes remains unexplained and several dermatologists who had been consulted were also unable to offer a satisfactory explanation.

In reference to the treatment it is worthy to note that a single exposure to roentgen-rays was followed by a shrinking of the substernal mass although no effect upon the cervical tumor was demonstrated. The patient's condition progressed so rapidly to a fatal issue that it was impossible to draw any conclusions as to the action of the radium seed. The tumor cells had already entered the circulation when we were first consulted as evidenced by the metastasis to the cervical glands and it is therefore extremely doubtful that X-rays or radium instituted at that time would have checked the condition. The most we could have hoped for would have been an amelioration of symptoms and a temporary remission in the progress of the growth.

No authentic cases of cures have been recorded in malignant thymoma. Groover, Christie, Merritt and Coe [14] reported a marked remission in the tumor in one case following deep radiation and an apparently complete and permanent disappearance in another but the nature of the tumor was not proved pathologically.

In reporting the data on this case of thymoma it is realized that no method of reasoning is offered whereby a correct diagnosis of this malignant neoplasm can be made clinically, for the ultimate decision will always rest with the pathologist. It is believed however that the publication of our experiences will in the occurrence of mediastinal neoplasms stimulate a closer search of the thymus gland as the possible origin of malignancy and thus lead to the recognition of such a tumor at a stage early in development when surgery or radiation may effect a cure.

Summary

The pathological studies on this case were made by Dr. Louise H. Meeker,[15] who summarizes the findings as follows:

All studies of thymoma end more or less in theoretical discussions with quotations from the authoritative works of Hammar,[16] Schridde,[17] Danchakoff [18] and Maximow.[19] The opposed views of these writers as to the genesis of the thymic cells accounts for the persistence of the indefinite term "thymoma." The rarity of "thymoma" accounts in part for our inadequate knowledge. According to our files the first record of thymoma in our hospital was in June 1926.

In this case we have a malignant tumor originating in the thymic region as evidenced by the clinical history and substantiated by the anatomical findings.

Grossly the tumor is white and leathery and has no sharp boundaries. It fills the entire mediastinum extending upward about the larynx, displacing the thyroid, ensheathing the large vessels of the neck and invading the cervical glands. Its lateral and downward extensions are directly along the pleura and pericardium following the vena cava and prevertebral connective tissue below the diaphragm and spreading to the viscera of the abdomen and pelvis. The abdomen and pelvic involvements include both kidneys, the stomach (intestine?) and ovary. Extension below the diaphragm is rare and no single previous thymoma reported has equalled the widespread involvement that has occurred in this case.

The striking features of the finer detail are first, the evident extension by way of the lymphatics and second, the pronounced infiltrating character of the neoplastic tissue especially shown by the obliteration of structural landmarks in the organs invaded. The radiated portions of the growth in particular show many areas of necrosis.

Microscopically the cells forming the tumor are of one general type growing diffusely throughout a scanty stroma although in some areas the stroma may be abundant. They are small cells for the most part with vesicular nuclei having one nucleolus and with scanty slightly basophilic or acidophilic cytoplasm. Closely intermingled with these just named cells there are large cells also with vesicular nuclei having nucleoli and more abundant and paler cyto-

plasm often with one to several processes. Many of the cells contain acidophile granules, still others are phagocytic and mitotic figures are occasionally fairly numerous and pyknotic forms are common. Irregular calcific deposits found only in sections from the true thymic region are interpreted as degenerated Hassal's corpuscles. We have interpreted the tumor cells as pretty closely resembling so-called small thymic cell and especially the thymic reticular elements. Hassal's corpuscles are not a part of the newgrowth.

REFERENCES

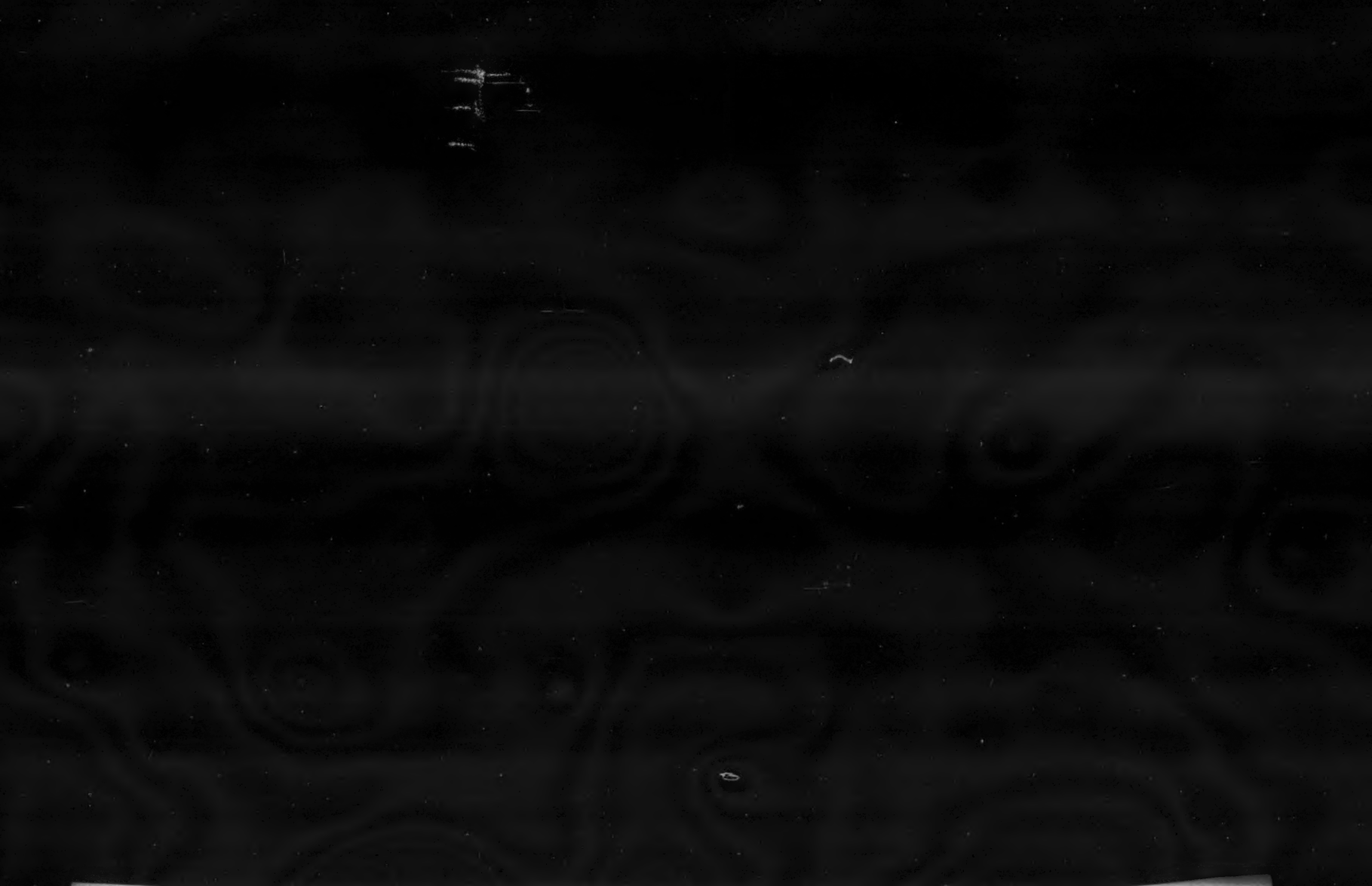
1. Ambrosini. Thèse de Paris, 1894.
2. Foot, N. C. Concerning malignant thymoma; with report on case of primary carcinoma of the thymus. *Am. J. Path.*, 1926, ii, 33.
3. Jacobson, V. C. Primary carcinoma of thymus. *Arch. Int. Med.*, 1923, xxxi, 847.
4. Symmers, D., and Vance, B. M. Epitheliomata of thymic origin. *Arch. Int. Med.*, 1921, xxviii, 239.
5. Ewing, J. Neoplastic Diseases, Ed. 3, 1928.
6. Zniniewicz, J. Inaug. Diss., Greifswald, 1911.
7. Strauss, S. G. Malignant neoplasms of thymus with report of case. *New York M. J.*, 1919, cx, 646.
8. Rubaschow, S. *Virchows Arch. f. path. Anat.*, 1911, ccvi, 141.
9. Friedlander, A., and Foot, N. C. Malignant small-celled thymoma with acute lymphoid leukemia, *Am. J. M. Sc.*, 1925, clxix, 161.
10. Foot, N. C., and Harrington, H. Malignant epithelial thymoma, report of case, with necropsy. *Am. J. Dis. Child.*, 1923, xxvi, 164.
11. Miller, J. A case of thymoma. *Canad. M. A. J.*, 1926, xvi, 810.
12. Helvestine, F., Jr. Malignant tumors of thymus. *Arch. Surg.*, 1924, ix, 309.
13. Baylis, A. B. Personal communication.
14. Groover, T. A., Christie, A. C., Merritt, E. A., and Coe, F. O. Roentgen-ray diagnosis and treatment of thymoma; report of two cases. *J. A. M. A.*, 1925, lxxxv, 1125.
15. Meeker, L. H. *Arch. Path. & Lab. Med.*, 1928, V, 928.
16. Hammar, J. A. "Morphology of Thymus." *Endocrinology*, 1921, v, 543.
17. Schridde, H. Thymus. Aschoff's Pathologische Anatomie II, Jena, Ed. 5, 1921, 176.
18. Danchakoff, V. Differentiation of cells. *J. Exper. Med.*, 1916, xxiv, 87.
19. Maximow, A. *Archiv. F. mikr. Anat.*, 1909, lxxiv, 525.

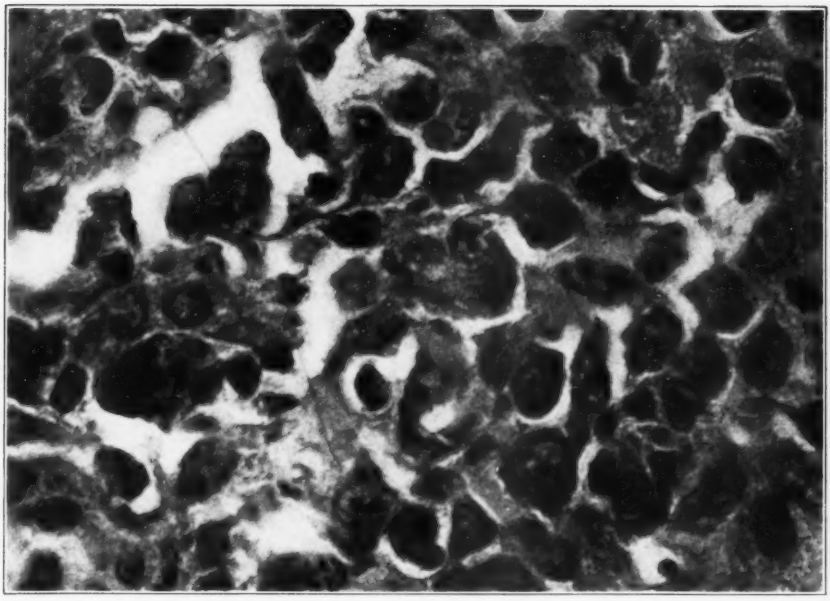
DESCRIPTION OF PLATES

Plate 10

Fig. 1. High power. The polymorphous reticular type cells are well shown.

Fig. 2. Photograph of gross specimen showing the masses attached to the sternum and the diffuse growth filling the mediastinum and extending over the pleura, pericardium and diaphragm.





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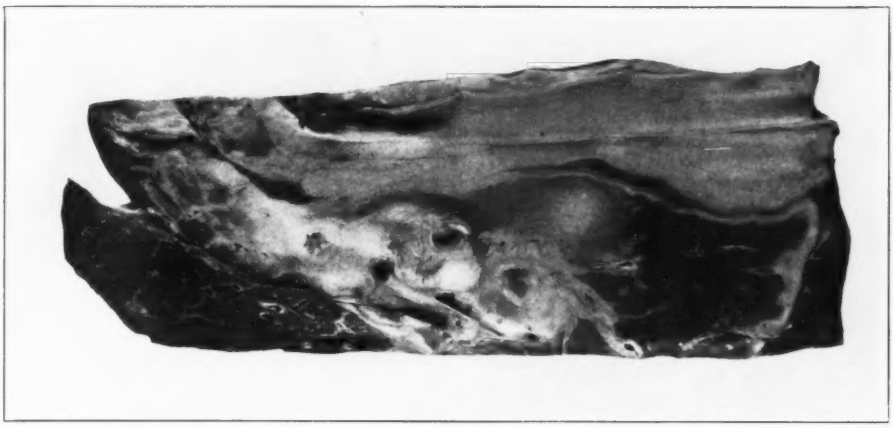
Herriman and Rahte Malignant Thymoma with Metastases

PLATE II

FIG. 3. Showing the pleural thickening by the tumor growth.

FIG. 4. Photograph of gross specimen. The entire heart is covered with a shaggy growth of tumor cells.



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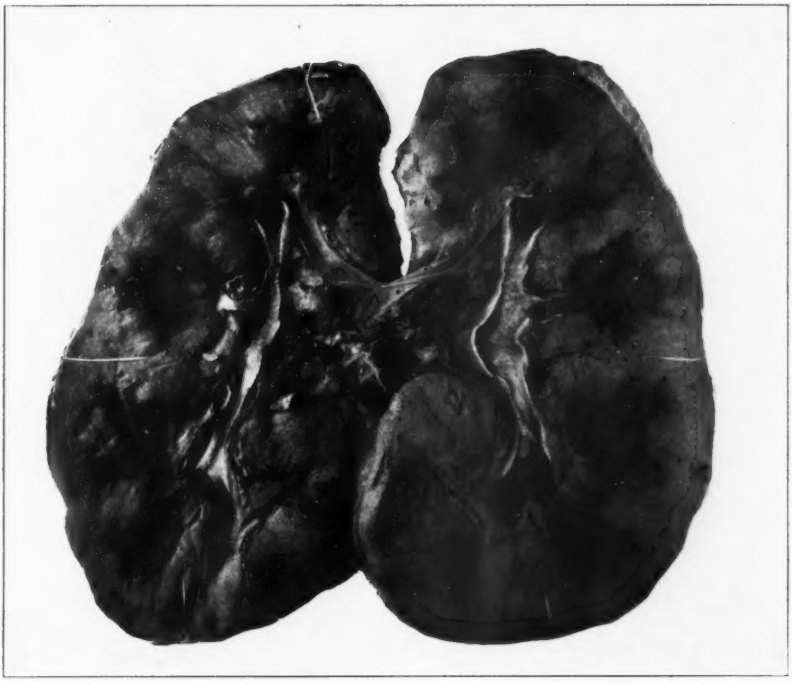
Herriman and Rahte Malignant Thymoma with Metastases

PLATE 12

FIG. 5. Photograph of kidney. All the pale areas are formed of tumor metastases.

FIG. 6. Photomicrograph of pericardium showing the thick mantle of tumor cells.



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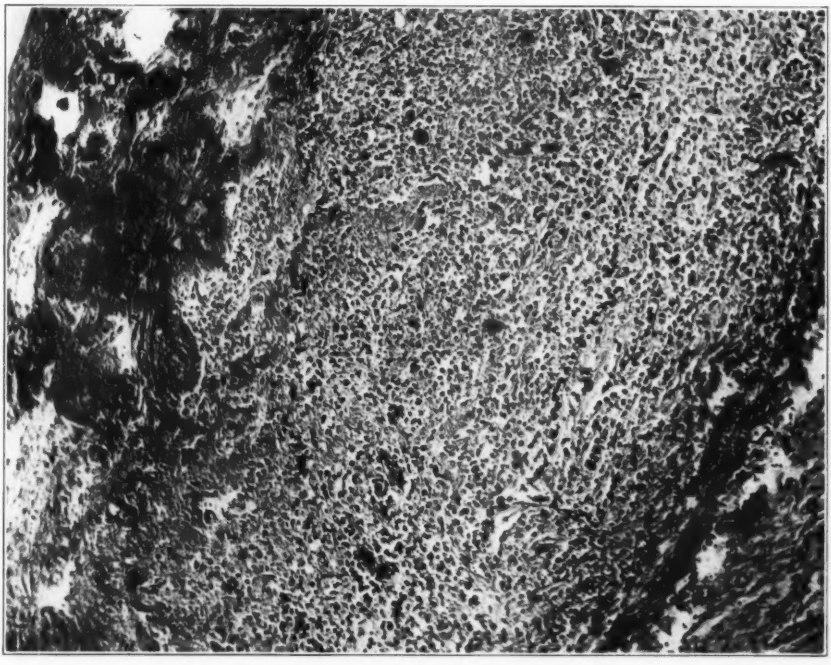

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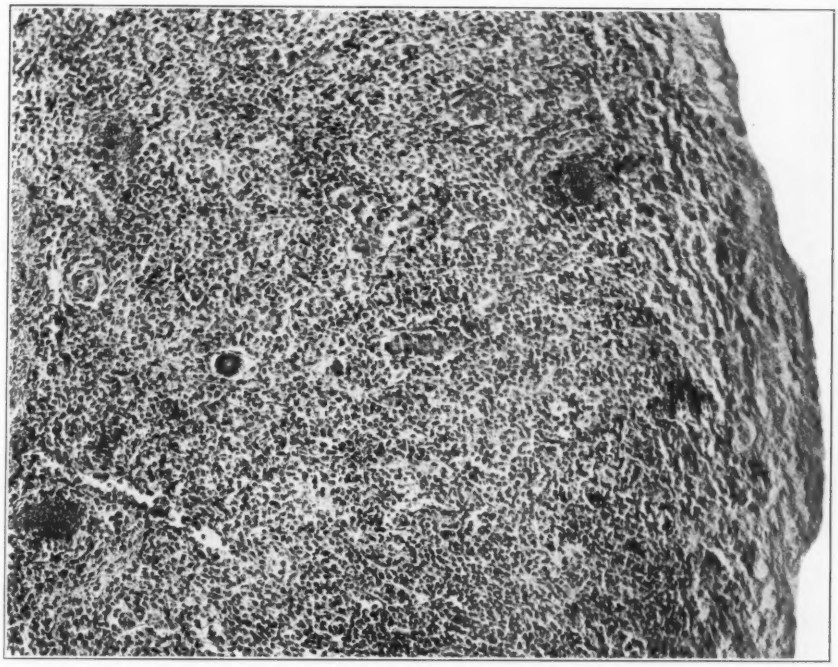
Herriman and Rahte Malignant Thymoma with Metastases

PLATE 13

FIG. 7. Photomicrograph of nodule in the mucous membrane of the stomach.

FIG. 8. Photomicrograph showing the invasion of the kidney by tumor cells.



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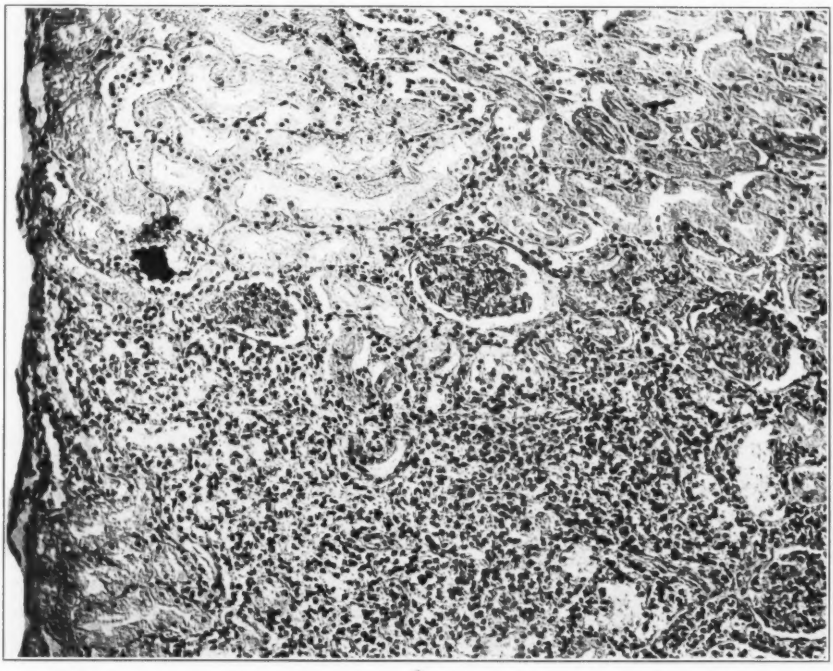

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Herriman and Rahte Malignant Thymoma with Metastases

THE INTIMAL LESION OF THE AORTA IN RHEUMATIC INFECTIONS *

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Rheumatic aortitis is now a well recognized entity and several instances have appeared in the literature. Although the first recorded observations on rheumatic arteritis date back to Bouillaud[1] in 1840, it was not until the work of Klotz [2] and Rabé [3] that accurate pathological data were collected on the subject. Klotz pointed out the flame-like scars about the nutrient vessels in the media and the cellular infiltrations of the adventitia. It is, however, to Pappenheimer and VonGlahn that we owe a complete histopathological description of the vascular lesions in rheumatic fever both in the aorta and the smaller vessels.[4, 5] In their first paper on the subject of aortitis in this disease [5] they found no intimal lesion but noted in addition to Aschoff bodies in the adventitia, dense and acellular scars in the media about the nutrient vessels. Here the initial lesion was described as a swelling, fragmentation, and lysis of the collagen fibrils. They subsequently [6] reported in a child 9 years of age with progressive recurrent rheumatic fever an instance of rheumatic aortitis with previously undescribed cellular infiltrations of the media as well as fairly typical changes in the adventitia. In the outer two-thirds of the media the vessels were thickened and about these there were polymorphonuclear leukocytes, lymphocytes, and larger elements having the structural and staining characteristics of "Aschoff cells."

More recently, the same authors reported two further instances of cellular rheumatic lesions in the aorta.[7] In one the intima was involved microscopically. In the other, accompanying typical changes in the endocardium of the left auricle and in the mesenteric, renal, and coeliac vessels, glistening brownish translucent patches of ridges were noted in the intima of each sinus of Valsalva and of the ascending aorta, resembling in many respects the endocardial lesions of the auricle. Microscopically the lesions were predomi-

* Received for publication July 17, 1928.

nately in the intima and the subjacent portions of the media. Bands of non-nucleated fibrillar tissue were found in the intima bordered by rows of deeply staining cells with basophilic cytoplasm and one or occasionally two large vesicular nuclei containing a central dense clump of chromatin. The intimal endothelium appeared swollen and polygonal and gave the appearance of migrating into the subjacent stroma. Occasional clefts were noted in the intima which in places appeared to communicate with the lumen of the aorta and were filled with red blood cells and bordered by polyhedral cells. No cholesterin crystals were found. The media showed a complete loss of muscle fibers over large areas with only collagen and elastic fibers persisting. In places the elastic coat was thinned out and ruptured. In the outer two-thirds of the media were seen perivascular infiltrations of the type previously described.*

These are not the only instances of intimal lesions of the aorta in rheumatism reported in the literature, although the few previously described cases are not unequivocal.** We are reporting two further instances of gross rheumatic involvement of the intima of the aorta.

CASE 1. *Clinical History:* No. 15817, M. P., male, age 10½ years, entered Montefiore Hospital on Feb. 5, 1928, with the complaint of palpitation and shortness of breath on exertion. During the preceding five years he had had recurrent attacks of rheumatic fever with evidence of cardiac involvement. On examination at the time of admission he presented the physical signs of mitral stenosis and insufficiency and aortic insufficiency. His blood picture was normal. During the greater part of his stay in the hospital he was febrile and developed a marked anemia. A few days before exitus he complained of severe precordial pain and a loud friction rub was heard over the apex and lower edge of sternum. The child died suddenly seven weeks after admission.

The autospy No. (4261) was performed 5½ hours after death.

The anatomical findings were: recurrent rheumatic endocarditis, mitral and aortic valves, and left auricle; mitral and aortic insuf-

* Kugel and Epstein [8] have recently reported microscopic rheumatic lesions of the pulmonary artery resembling the aortic lesions described by Pappenheimer and Von Glahn. They noted the frequency of involvement of the pulmonary musculo-arterial junction.

** A complete review of the literature bearing on lesions of the blood vessels in rheumatic fever may be found in the publications of Pappenheimer and VonGlahn. Chiari [9] has recently described six instances of chronic rheumatic infection with adventitial lesions of the aorta and its main branches. These consisted of perivascular infiltrations with "Aschoff cells," plasma cells and lymphocytes.

ficiency; cardiac hypertrophy and dilatation; rheumatic aortitis; organizing serofibrinous pericarditis; partial atelectasis left lung; chronic passive congestion abdominal organs; ascites.

Heart: The heart weighed 600 gm. It was covered by a shaggy exudate. The mitral and aortic valves presented the typical changes of recurrent rheumatic valvulitis. In addition to an extremely rich vascularization of the aortic leaflet of the inflamed mitral valve, there were two other interesting findings. One was the presence in the endocardium of the left ventricle, about 0.5 cm. below the right anterior aortic cusp, of an oval slightly raised patch about 1.5 cm. in diameter and 1 to 2 mm. in thickness, consisting of soft, yellowish gray tissue with a finely striated surface. It was well demarcated from the surrounding endocardium by a serrated margin. There was a similar plaque, 0.5 cm. in diameter, on the ventricular surface of the aortic leaflet of the mitral valve about 8 mm. below the junction of the posterior and left anterior aortic cusps. The other interesting finding was the presence of several transverse ridges in the thickened endocardium of the dilated and hypertrophied left auricle.

Aorta: The aorta (Fig. 1) on its intimal surface about 0.5 cm. above the commissural junction of the posterior and right anterior cusps presented a well demarcated oval plaque about 1 by 0.7 cm. in size and raised about 1 mm. Grossly it involved only the intima. The surface had a pale brownish yellow color, and in the central portion well defined longitudinal furrows were seen. About 0.5 cm. above the commissure between the posterior and left anterior cusp was a similar plaque about 7 mm. in diameter presenting an irregular, markedly injected, shallow central depression. A third plaque of this nature, 4 mm. in diameter, about 0.5 cm. above the margin of the left anterior sinus of Valsalva, was removed for microscopic study. There were a few simple atheromata of the intima in the ascending and lower abdominal portions of the aorta, extending for a short distance into the common iliacs. The other large arteries and the coronary arteries were grossly normal.

Microscopical Findings

Left Auricle: In a section taken just above the mitral valve the endocardium is markedly thickened. Just beneath the endothelium is an accumulation of fibrin. Beneath this the tissue is infiltrated with numerous round cells and elongated, deeply basophilic connec-

tive tissue cells containing spindle-shaped nuclei, and arranged in horizontal orientation to the surface. Sections through the thickened ridges noted in the gross show a marked cellular infiltration. In part this consists of lymphocytes and peculiar comma-shaped cells arranged in places in a vertical orientation to the surface on either side of bands of swollen collagen fibers. In the depths of the endocardium is a diffuse infiltration with polymorphonuclear leukocytes and lymphocytes, continuous with a similar exudate in the subjacent myocardium, in places following the course of blood vessels. The myocardium itself shows a moderate hypertrophy of the muscle fibers and a diffuse fibrosis, chiefly perivascular. In this connective tissue are seen a few round cells and an occasional "Aschoff cell."

Left Ventricle: The endocardium is thickened and infiltrated with numerous round cells and epithelioid cells with basophilic cytoplasm and large vesicular nuclei. These appear very much like "Aschoff cells." Other sections through the ventricular wall show an organizing exudate in the thickened pericardium with accumulations of round cells, leukocytes, and fibroblasts, some with basophilic cytoplasm and large vesicular nuclei. In the myocardium are perivascular accumulations of lymphocytes, plasma cells and occasional polymorphonuclear leukocytes and large "Aschoff cells."

Pulmonary Artery: (musculo-arterial junction). The artery itself shows no lesions of the wall, but at the myocardial junction there are perivascular accumulations of round cells, polymorphonuclear leukocytes and "Aschoff cells."

Coronary Artery: A section shows only a low-grade atheromatosis.

Aorta

A section through the plaque (Figs. 2, 3, 5, 6) shows a striking picture. There is a loss of the lining endothelium and the intima is replaced by a broad layer of fibrin. Beneath this layer is a zone of connective tissue rich in large dilated capillaries and containing numerous cells arranged for the most part with their long axes perpendicular to the surface, in places grouped about capillaries. They are large, irregular, spindle-shaped, connective tissue cells and in one area invade the fibrin zone, penetrating to the free surface of the intima. The greater portion of the exudate in its outer third contains numerous small pyknotic distorted nuclei, the exact nature of which cannot be determined. Some are elongated and arranged

vertical to the surface. At the periphery of the plaque the fibroblasts have reached the surface of the exudate forming a closely packed layer in which the long axes of the cells are arranged parallel to the surface. In addition there are in this area a number of peculiar irregular, elongated, spindle-shaped cells with large, irregular, oval, sometimes slightly lobulated, vesicular nuclei, some of which show pale basophilic spherules as well as occasionally a prominent nucleolus. The cytoplasm is deeply basophilic and indistinctly granular. In some sections through the organized portions of the exudate there are deep and irregular narrow endothelial-lined clefts communicating with the lumen of the aorta. The intima of the adjacent aorta where no fibrin exudate is present is very cellular, containing numerous closely packed cells of the type just described. These cells were seen also in the pericardial organization tissue described above, and we have seen identical cells in the granulation tissue of a non-rheumatic organizing fibrinous pericarditis. Although they sometimes approximate the "Aschoff cells" in appearance they are most probably actively proliferating fibroblasts. A few lymphocytes and an occasional polymorphonuclear leukocyte are also seen. No plasma cells or giant epithelioid cells of Aschoff are present. With Weigert's elastic tissue stain it is seen that the fibrin exudate is almost entirely within the limits of the internal elastic membrane, which is fragmented and frayed out.

Media: In the inner portion of the media and at the site of the granulation tissue, the elastic fibers show extensive distortion, destruction, fragmentation and fraying. The nutrient vessels penetrate the outer third of the media and in an area corresponding to the extent of the intimal exudate they are surrounded by peculiarly radiating cells lying in a pale ground substance. These cells (Fig. 4) seem to sweep downward in the form of a fan toward the intimal surface and are most numerous along the wide flare of the "fan." The cells, although somewhat irregular in size, are uniformly spindle-shaped with dark staining large oval nuclei and deeply basophilic cytoplasm. There is an occasional polymorphonuclear leukocyte. The ground substance of the fan-like areas consists of a delicate, fibrillar, very pale staining connective tissue containing several small capillaries in addition to the main nutrient vessel. The elastic tissue in these areas shows extensive destruction, fragmentation, clumping, and fraying. A most striking feature is

that these fan-like areas are present only about the nutrient vessels corresponding to the area of intimal exudation. The remaining nutrient arteries show smaller areas of cellular infiltration with round cells, a few proliferating fibroblasts and leukocytes, and several groups of two or three round and oval wandering cells with homogeneously dark staining round nuclei and deeply basophilic non-granular cytoplasm.

Adventitia: Throughout the adventitia, frequently perivascular, are numerous small cellular infiltrations made up chiefly of lymphocytes, a few large wandering cells and an occasional Aschoff giant cell. The scattered, large, wandering cells have oval dark staining nuclei and show extreme variations in shape, many having long protoplasmic processes. Several have distinctly granular cytoplasm not unlike that of mast cells.

Thoracic and Abdominal Aorta: Sections from the thoracic and abdominal portions of the aorta show the characteristic mild perivascular infiltrations limited to the adventitia which have been described by Pappenheimer and VonGlahn.[4] They are made up chiefly of round cells, but there are also present a few plasma cells and in places a few leukocytes and an occasional mast cell. In several instances these infiltrations surround large multinucleated cells, typical of Aschoff giant cells.

Smaller Arteries: The smaller arteries in various portions of the body also show evidence of involvement. In one of the mesenteric arteries in an area just beneath the endothelium there are seen round cells and a few distorted cells with elongated nuclei, whose identity cannot be established. In the surrounding fat is an extensive infiltration of round cells and polymorphonuclear leukocytes. A few plasma cells are present, but no definite "Aschoff cells." Similar infiltrations are seen in the fat surrounding the small vessels throughout the section. In the hilum of the spleen about some of the small arteries there are small accumulations of round cells and an occasional leukocyte but no "Aschoff cells." In the section of kidney adjacent to the wall of a vein there is an accumulation of round cells and an occasional plasma cell. In the perisuprarenal fat adjacent to the small vessels are seen accumulations of leukocytes and several epithelioid and multinucleated giant cells, whose cytoplasm, however, takes only a faintly basophilic tint with the Pappenheim stain. No acute fibrinous lesions of the wall such as described

by Pappenheimer and VonGlahn [5] were noted. Pappenheim stains of sections of the aorta as well as the other organs failed to reveal the presence of bacteria.

CASE 2. *Clinical History:* No. 04369, A. L., female, aged 16 years, admitted to Montefiore Hospital on Nov. 18, 1920, with the complaint of dyspepsia, precordial distress, weakness, and pains in the joints for a period of about fourteen years. These symptoms began following an attack of scarlet fever at the age of three, and had since then recurred at irregular intervals. On examination the patient was moderately anemic and presented the physical signs of mitral and aortic stenosis and insufficiency with cardiac hypertrophy. The liver and spleen were palpable. There was no edema or ascites. During her stay in the hospital she developed an acute exacerbation of her rheumatic infection with cardiac involvement following an attack of tonsillitis. She recovered, however, and remained ambulatory and in fairly good condition for four months before exitus. She died suddenly during sleep, ten months after admission.

The autopsy (No. 3473) was performed twenty-eight hours postmortem by Dr. D. P. Seecof.

The anatomical findings in brief were: recurrent rheumatic endocarditis, mitral and aortic valves, left ventricle; mitral stenosis and insufficiency; aortic stenosis and insufficiency; rheumatic myocarditis; rheumatic aortitis; chronic adhesive pericarditis; healed mural thrombus, right auricle; chronic passive congestion of viscera.

Heart: The heart was markedly enlarged, the pericardial cavity obliterated by thin sheet-like fibrous adhesions. On section was found an advanced, in part calcified, in part ulcerated, rheumatic involvement of the mitral and aortic valves, as well as slight thickening of the pulmonic and tricuspid leaflets. The left auricle and ventricle were hypertrophied and dilated, the left ventricular wall measuring 20 mm. The musculature contained numerous pearly white scars. In the region of the interventricular septum, immediately below the aortic ring, the intima of the left ventricle was the site of an irregularly thickened, fibrous, flattened elevation, 2 cm. in diameter and 1 to 2 mm. in thickness.

Aorta: In the ascending portion of the aorta was an irregular, large, brownish yellow, flattened plaque similar in appearance to the one described in the left ventricle.

MICROSCOPICAL FINDINGS

Heart: The heart shows numerous perivascular scars which in places are infiltrated with round cells and multinucleated cells of

the Aschoff type. The muscle fibers are hypertrophied. At the site of the endocardial plaques in the left ventricle the endocardium is thickened and contains sparsely cellular, partly hyalinized, edematous connective tissue, in which no characteristic elements can be discerned.

Aorta

Immediately beneath the endothelium, the intima and adjacent inner portion of the media are irregularly thickened (Fig. 7) forming a prominent elevation which presents deep endothelial-lined clefts communicating with the lumen of the aorta. Just beneath the endothelium at the peripheral margin of the plaque are numerous spindle-shaped cells arranged parallel to the surface and having the appearance of connective tissue cells. In addition, there is a moderate number of round and oval cells with round and oval, homogeneously dark staining nuclei and strongly basophilic cytoplasm, as well as an occasional leukocyte. In the intima, limited by the internal elastic membrane, there is a peculiar pale staining, finely fibrillar tissue containing a few scattered capillaries and diffusely infiltrated with a moderate number of lymphocytes and many fibroblasts, some having more or less basophilic cytoplasm. The fibrillar material stains as fibrin with Mallory's phosphotungstic acid hematoxylin.

Media: In the portion of media comprising the deeper layers of the plaque are seen diffusely scattered connective tissue cells lying in small lacunae in a stroma of swollen fibrillar tissue, which in places is highly vascular. Just external to the area of greatest vascularization is a very dense infiltration with irregular, closely packed, oval and spindle-shaped connective tissue cells, some with basophilic cytoplasm, the long axes of which are arranged perpendicular to the intimal surface. Among these are scattered several large cells with intensely basophilic staining cytoplasm and multiple or lobulated dark staining and vesicular nuclei, "Aschoff cells." A few round cells with dark staining homogeneous nuclei and basophilic cytoplasm are present but no definite plasma cells. In the region of these dense cellular infiltrations the Pappenheim stain reveals numerous small particles of basophilic staining material. The elastic tissue of the internal elastic membrane is in places thinned out and fragmented. The elastic tissue of the media which is included in the plaque, particularly where the latter is thickest, is

extensively fragmented, frayed and distorted. There is a very striking loss of muscular elements in the remainder of the media at the site of the plaque. The nutrient vessels penetrate as deeply as the middle of the media and are accompanied by small perivascular accumulations of lymphocytes, occasional leukocytes, plasma cells and a scattering of mast cells. A few of the latter have nuclei characteristic of those of the plasma cells. In this outer, vascularized portion of the media are seen striking destructive changes in the elastic tissue in addition to a disappearance of the muscle fibers over a large area corresponding to the site of the intimal plaque.

Adventitia: The adventitia is highly vascular; the connective tissue is increased and has an edematous appearance. About many of the capillaries there are fairly dense cellular accumulations, consisting chiefly of lymphocytes and plasma cells. A few of the latter show transition forms to plasma mast cells. There are also a few "Aschoff cells" among these infiltrations.

Sections of the remaining organs show evidences of marked chronic passive congestion.

Discussion

In a consideration of the various types of lesions described in the aorta on rheumatic fever, three groups may be distinguished:

1. Involvement of the adventitia alone, with perivascular infiltration and the formation of Aschoff bodies (Fig. 4).

2. Involvement of the adventitia and the media. The lesion at the latter site may be in the form of (*a*) an acute, cellular, perivascular infiltration in the outer third with destructive changes in the media immediately around the vessels (Fig. 6); or (*b*) perivascular scars of longer standing at this site, the consequent interference with the blood supply resulting in a patchy loss of muscle cells in the remainder of the media (Fig. 4).

3. Involvement of all three layers of the aorta with either (*a*) an acute process, as in Case 1, in the form of a fibrinous intimal exudate accompanied by cellular infiltrations about the nutrient vessels in the outer third of the media with perivascular destructive change in this region; or (*b*) a lesion of longer standing as in Case 2, consisting of an organized intimal plaque accompanied by characteristic infiltrations about the nutrient vessels, with extensive muscular atrophy throughout the corresponding portions of the media.

In view of the strict gradation which can be discerned among these various types of lesions, we are inclined to believe that they represent rheumatic infections of the aorta by way of the vasa vasorum, the variations in the picture depending upon the severity of the infection and the chronicity of the lesion. We do not think that the intimal lesion is to be considered as a direct infection from the intimal surface.

Summary

Two instances of macroscopic involvement of the aorta in recurrent rheumatic fever are described. A striking feature, which we believe has not been previously described, is the presence in one of the cases of an acute fibrinous lesion of the intima. In brief, the characteristics of the lesion are:

1. Aschoff bodies in the adventitia.

2. Perivascular (in the acute stage, fan-like) infiltrations in the outer third of the media, with destruction of elastic tissue and muscle elements.

3. Recent and organized fibrinous plaques in the intima, the connective tissue cells comprising the vascular organization tissue having a characteristic vertical orientation at the base of the intimal lesions.

REFERENCES

1. Bouillaud. J. B. Traité clinique du rhumatisme articulaire aigue. 1840. (*Cf.* Pappenheimer and VonGlahn, Ref. 4.)

2. Klotz, O. Rheumatic fever and the arteries. *Tr. Assn. Am. Phys.*, 1912, xxvii, 181.

3. Rabé. Contribution à l'étude des lésions des artères dans l'infection rhumatismale. *Presse Méd.*, 1902, ii, 927. (*Cf.* Ref. 4.)

4. Pappenheimer, A.M., and VonGlahn, W. C. Lesions of the aorta associated with acute rheumatic fever and with chronic cardiac disease of rheumatic origin. *J. Med. Res.*, 1924, xliv, 489.

5. VonGlahn, W. C., and Pappenheimer, A.M. Specific lesions of peripheral blood vessels in rheumatism. *Am. J. Path.*, 1926, ii, 235.

6. Pappenheimer, A. M., and VonGlahn, W. C. A case of rheumatic aortitis with early lesions in the media. *Am. J. Path.*, 1926, ii, 15.

7. Pappenheimer, A. M., and VonGlahn, W. C. Studies in the pathology of rheumatic fever. Two cases presenting unusual cardio-vascular lesions. *Am. J. Path.*, 1927, iii, 583.

8. Kugel, M. A., and Epstein, E. Z. Lesions in the pulmonary artery and valve associated with rheumatic cardiac disease. *Arch. Path.*, 1928, vi, 247.

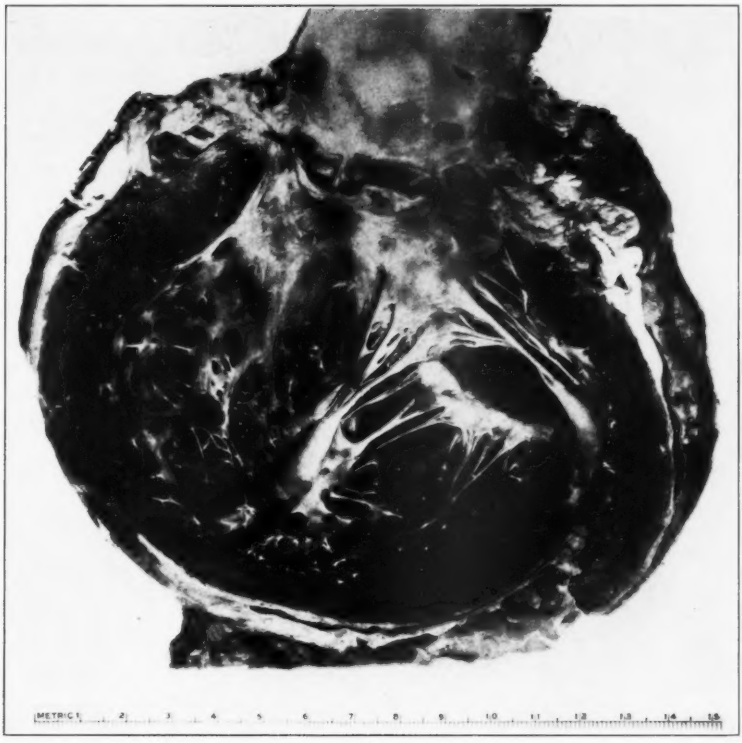
9. Chiari, H. Über Veränderungen in der Adventitia der Aorta und ihrer Hauptäste im Gefolge von Rheumatismus. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1928, lxxx, 336.

DESCRIPTION OF PLATES

PLATE 14

FIG. 1. Case 1. Heart and aorta. Showing two of the three aortic plaques.

FIG. 2. Case 1. Aorta. H and E. Showing low power view of intima and media. Marked fibrinous infiltration of intima and perivascular cellular medial infiltration. × 40.



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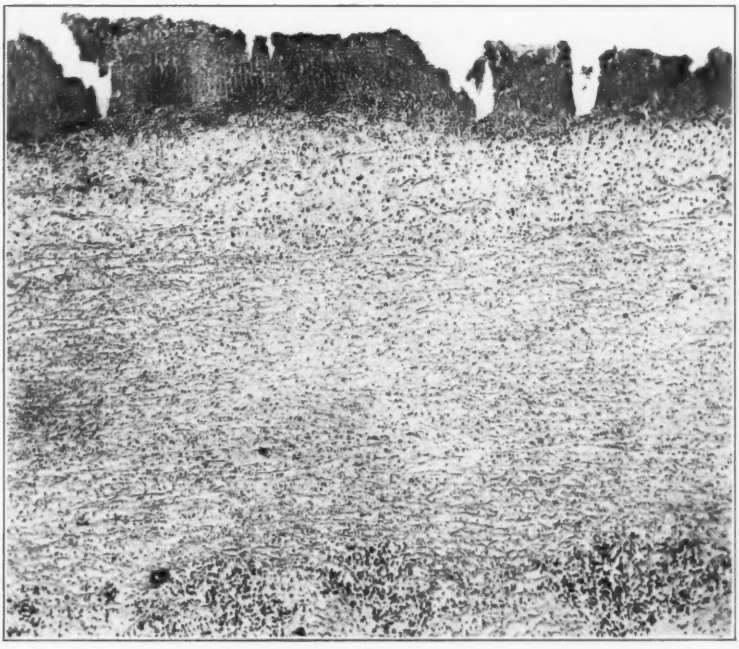

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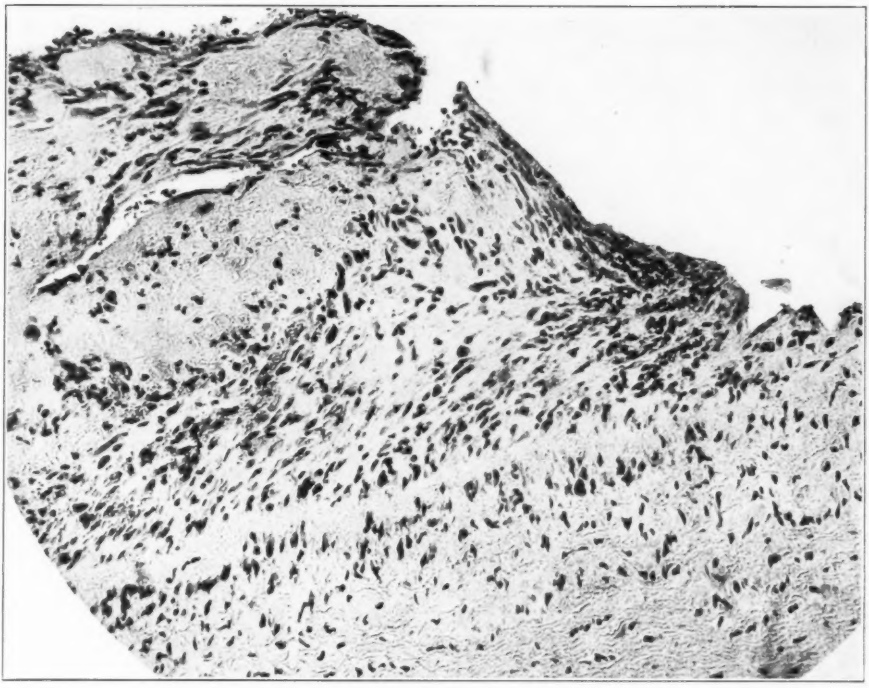
Plate 15

Fig. 3. Case 1. Aorta. H and E. Organized periphery of an intimal plaque showing characteristic endothelial-lined indentation. × 200.

Fig. 4. Case 1. Aorta. H and E. Showing the fan-like medial infiltration. × 200.



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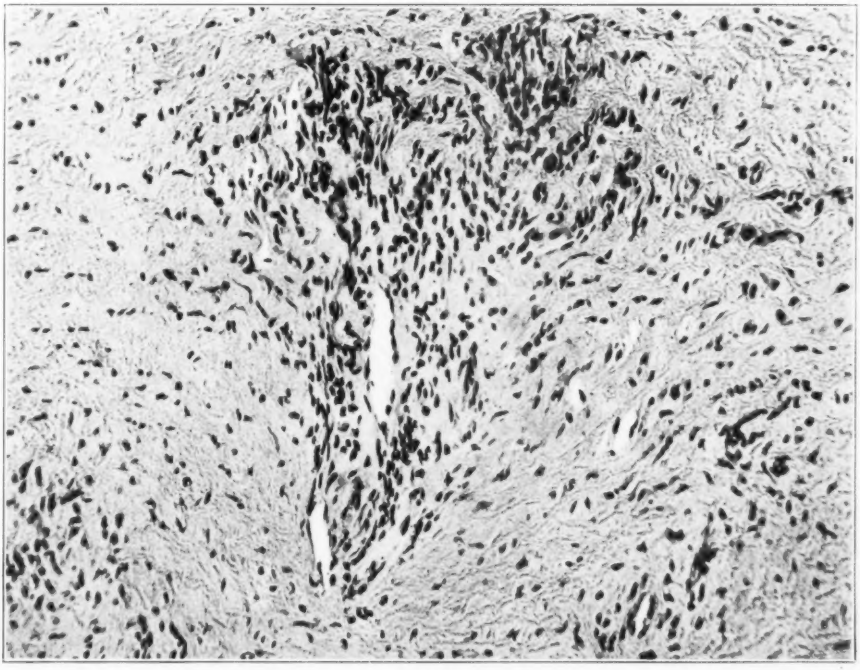

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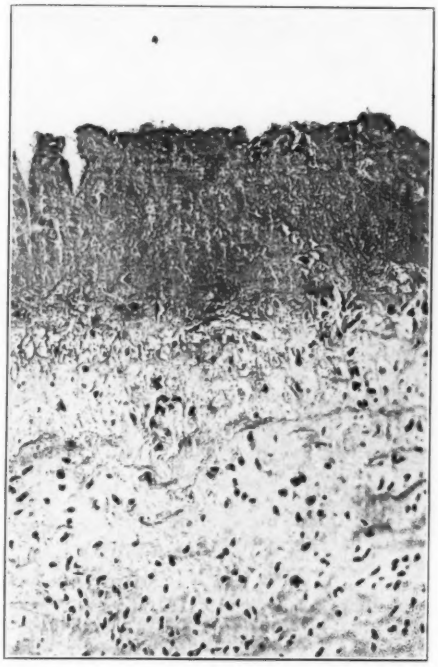
Plate 16

Fig. 5. Case 1. Aorta. Showing the high fibrin content of the intimal plaque and the cellular elements of intima growing into the fibrin plaque. × 40.

Fig. 6. Case 1. Aorta. Weigert's elastic tissue — Van Gieson. Extensive destructive changes in elastic tissue. × 40.

Fig. 7. Case 2. Aorta. H and E. Showing the organized intimal plaque presenting intimal indentations and cellular infiltration at base of plaque. × 40.



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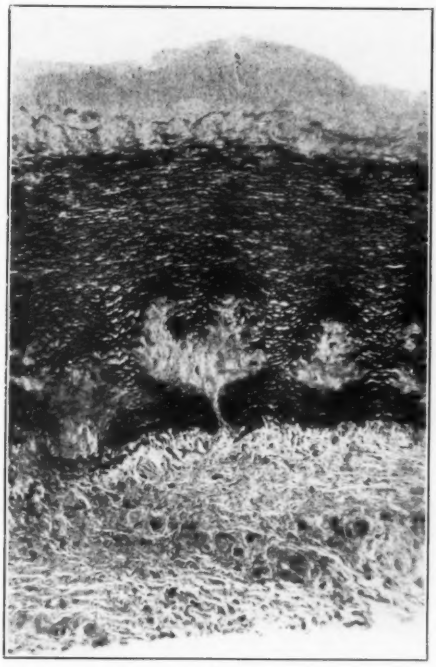

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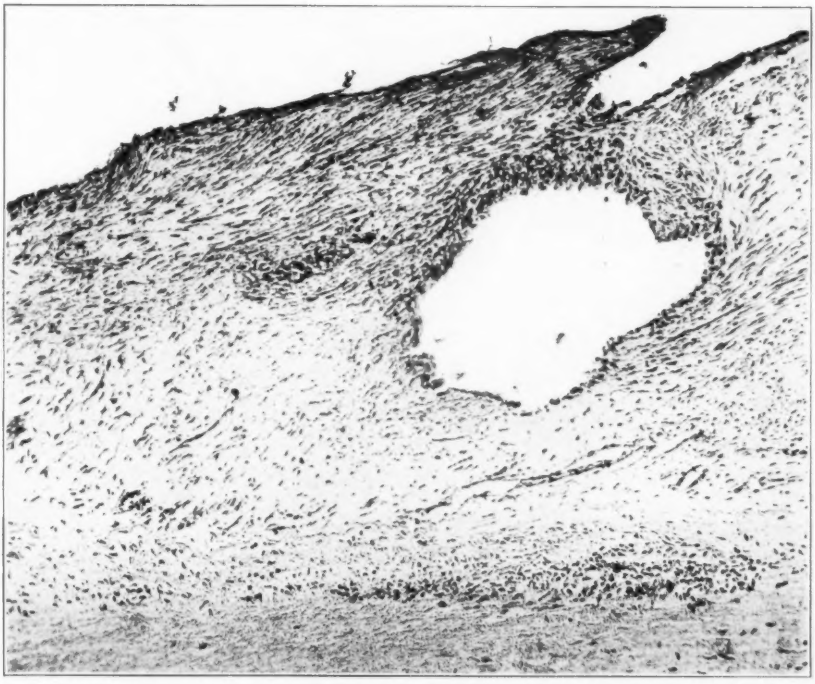

7

Perla and Deutch Lesion of Aorta in Rheumatic Infections

TISSUE CHANGES ASSOCIATED WITH VITAMIN *A* DEFICIENCY IN THE RAT *

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The conception of the vitamins as specific dietary requirements rests in part upon the uniformity and reproducibility of the effects resulting from a lack of these substances in the food. Not only are food intake and growth affected but other alterations in physiology and structure can be demonstrated. It was early pointed out by Osborne and Mendel (1913) [1] that when rats are given a simple ration, adequate except for vitamin A, there ultimately develops a type of eye disease which may be cured by incorporating butter fat in the food. This was long considered the principal lesion associated with this dietary deficiency.

In a further study of the changes associated with vitamin A deficiency Mori (1922) [2] concluded that the principal lesion is an atrophy of the glandular epithelium and a loss of secretory power on the part of the lacrymal gland. He stated that the drying of the eye and the xerosis of the corneal epithelium (xerophthalmia) are entirely dependent upon the lack of glandular activity. This investigator also found cornification of the mucosa of the larynx and trachea and of the ducts of the submaxillary, sublingual and parotid glands to be present. He said that if two per cent cod liver oil be added to the diet of rats with ophthalmia the conjunctival sac becomes moistened and the xerosis disappears. At the same time the lacrymal and salivary glands become normal histologically and show evidence of secretion. In contrast to the above are the papers reported by Yudkin and Lambert (1923).[3, 4] These authors concluded that the ocular manifestations of vitamin A deficiency are dependent upon the presence of a low-grade inflammatory process which commences in the nictitating membrane and palpebral conjunctiva, and spreads to the cornea, and that the keratinization of the corneal epithelium is

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secondary to the inflammatory process. Infection, according to them, plays an important rôle. Wason (1921)[5] concluded that the corneal changes are due to infection. She found no pathological changes other than those in the eye.

Wolbach and Howe (1925)[6] have made an extensive study of the pathological changes throughout the body of the rat. They concluded that the primary change is in epithelium and comprises "the substitution of stratified keratinizing epithelium for normal epithelium in various parts of the respiratory tract, alimentary tract, eyes and the paraocular glands and the genito-urinary tract." They did not state definitely in what order the above systems are involved, though in their experience the epithelium of the turbinate bones and of the submaxillary gland is always affected early. They mentioned the formation of large cysts in the accessory glands at the base of the tongue and described them as being lined by keratinizing epithelium. The cysts often attain a large size, sufficient to interfere with swallowing. They did not say in what proportion of cases this lesion occurred, but they believe that the process of keratinization and cyst formation can take place in the absence of infection. The submaxillary gland has received more attention from these authors and it is their opinion that changes in this structure precede those in the eye. They concluded that the lack of secretion of the glands throughout the body does not play a primary rôle in producing the metaplasia; that infection also is a secondary manifestation; and that the lack of the single vitamin A factor is directly responsible for the metaplastic activity of the epithelium.

It has been pointed out by Goldblatt and Benischek (1927)[7] that the diets employed by Mori, and by Wolbach and Howe, were deficient in vitamins D and C as well as vitamin A. Goldblatt and Benischek, however, carried on experiments of their own and were able to reproduce the lesions described by the previous authors when vitamin A alone was lacking in the diet. They also noted the great frequency of abscesses at the base of the tongue in rats which were on a deficient diet and believed that the infectious process is "coincident with or subsequent to the epithelial metaplasia and is not responsible for the initiation of the changes." No metaplastic changes were found by them in other structures when there was none in the sublingual glands and respiratory tract. Tongue lesions have also been noted by Sherman and Munsell (1925)[8] who found

them in seventy-six per cent of rats on a diet deficient in vitamin A. The antirachitic potency was not considered in their experimental diet. According to Osborne and Mendel (1917)[9, 10] and Van Leersum (1928)[11] there is a high incidence of urolithiasis in rats deprived of vitamin A. In Van Leersum's experience accompanying infection of the kidney is uncommon.

All workers thus far are in accord in finding epithelial metaplasia to be present in vitamin A deficiency. There is still some question about the rôle of infection in the early stages of the epithelial transformation. A satisfactory correlation of the appearance of xerophthalmia with the occurrence of lesions elsewhere in the body has not been made. The order in which the several parts of the body are affected has not been established, and the sequence of events, if the deficient diet is corrected, has not been adequately described. It is therefore of interest to investigate these phases of the question with a view to correlating the sequence of events with respect to the age and weight of the animal, the relation of xerophthalmia to the appearance of other lesions in the body and the effect of correcting the deficient diet.

Experimental Procedures

Animals and Diet: White (albino) rats were used in this experiment. They were of the strain of the Connecticut Agricultural Experiment Station and were bred in the laboratory. The special diets were started as soon as the animals were at the age of 21 to 24 days. The rats were kept in individual wire cages which were supplied with false bottoms, thus preventing coprophagy. The cages and food cups were kept scrupulously clean and each cage was sterilized with steam once a week.

Individuals from each litter were divided into four groups. Group I comprised animals which were to be observed early in the course of the experiment. In Group II were those which were to be allowed to develop outspoken vitamin A deficiency or to die of the disease. The animals in Group III were to develop symptoms of vitamin A deficiency and were then to be placed on a corrected diet. Group IV comprised the positive control rats. All animals were weighed at least twice a week and sometimes every day. The food intake was determined every four days.

The diet consisted of the following:

Extracted Casein *	18 per cent
Cornstarch	51 per cent
Crisco	27 per cent
Salt Mixture (Osborne and Mendel)	4 per cent

The Crisco was melted and the other constituents thoroughly mixed with it. When allowed to cool the food was of a pasty consistency which prevented spilling. This mixture was given the rats *ad libitum*. Vitamin B was provided by giving 200 mg. daily of dried yeast which was placed in a separate dish. For vitamin D a quantity of dried yeast was irradiated under a mercury vapor lamp for one-half hour at a distance of one foot, Hess (1927).[12] Two hundred milligrams of this were fed every day. Vitamin C was lacking in the diet but it has been shown by Parsons (1920),[13] by Parsons and Hutton (1924),[14] and by Lepkovsky and Nelson (1924)[15] that the absence of this factor in the diet has no effect on the rat. The drinking water was distilled. In addition to this diet the animals in Group IV received 10 drops of cod liver oil (Squibb) each day.

Histological Technique: All tissues were fixed in 10 per cent formalin and mounted in paraffin. Hematoxylin and eosin stains were made routinely and in addition Gram and connective tissue preparations were used occasionally.

The accessory salivary glands at the base of the tongue, the submaxillary gland, the trachea and bronchi and the renal pelvis were studied in all cases. The base of the tongue has received particular attention as the lesions occur more uniformly in this region, and because of its size it is possible to make a more complete study of the tongue. Accordingly, five blocks of the tongue were mounted in each case, and from each of the blocks two to six microscopic sections were made. From the other areas one or two blocks were mounted.

Results

Clinical Course and Gross Findings: The animals on the deficient diet gained steadily for a time but always less rapidly than the control rats in the same litter. Xerophthalmia appeared on an average when the rat had been on the diet forty days, though the extremes

* 95 per cent alcohol for 2 hours; ether, 2 hours; ether, 1 hour. Dried in oven at 105° C for 48 hours.

were twenty-seven and fifty days. The sequence of events in the development of xerophthalmia were: first, a slight swelling of the eyelids accompanied by photophobia (slit-like eyes); second, a clear discharge from the eyes which sometimes became blood-tinged; third, drying of the eyes, the lids becoming glued together and the eyeball appearing to sink into its socket. Occasionally corneal ulceration and hypopyon developed. Anterior staphyloma occurred in one case.

The appearance of xerophthalmia in relation to weight loss was variable. Sometimes eye changes appeared before there was any loss of weight. Again, several grams of body weight would be lost before eye changes became manifest. Occasionally xerophthalmia appeared and the body weight continued to increase and reached a maximum level several days later. The most usual sequence was an increase in weight to a point where it remained stationary for two or three days, then, appearance of xerophthalmia and rapid decline in weight. In Group I the animals were sacrificed early in the course of the deficiency. Some were killed when a few grams of weight had been lost, others when weight had been lost and the very earliest eye changes were observed. Rat 12 was killed before any changes were observed and Rat 26 had had xerophthalmia for six days when it was sacrificed. At autopsy no gross lesions were noted in the animals in this group. In Group II all the animals had varying degrees of xerophthalmia. All showed great emaciation and every rat had abscesses at the base of the tongue from which pus could be expressed. The latter lesions probably cause pain on swallowing and may account for the terminal falling off of food intake as is suggested by Goldblatt and Benischek. The tongue and eye lesions were the only constant gross findings. The submaxillary gland was often atrophied and occasionally contained abscesses. Urinary calculi were found in some cases and where obstruction occurred pyonephrosis was present.

The animals in Group III were allowed to develop xerophthalmia. and after several days when the eye changes were outspoken and considerable weight had been lost, cod liver oil was given. Often the rats were so weak that the oil had to be given by dropper. As a rule the response to diet correction was rapid. The xerophthalmia disappeared in from one to five days and the weight curve rose abruptly. Half the animals planned for this group could not be used as compli-

cations caused the death of the rat in spite of treatment. However, Rat 7 gained weight rapidly and was apparently well when autopsy revealed an extensive unilateral pyonephrosis. Rat 18 was allowed to develop xerophthalmia twice. The second time cod liver oil did not cure, as a pyonephrosis and perinephritic abscess were present. Following cod liver oil administration the rats were sacrificed at varying intervals. In the oldest cases no gross lesions were visible except in Rat 7. In those killed soon after cod liver oil was given, tongue lesions were present.

When cod liver oil was provided from the start (Group IV) the animals gained weight rapidly, and showed no gross evidence of disease whatever even after as much as one hundred and forty-four days of the diet.

Histological Findings: The earliest changes (Table I) are found in the salivary glands at the base of the tongue. These changes consist in dilatation of the main ducts with metaplasia of the duct epithelium to the squamous keratinizing type. Infection is always present even in the earliest detectable lesion and is often found in parts of the tongue where keratinization has not appeared. The infection is characterized by a polymorphonuclear leukocytic exudate and by necrosis of the acinar epithelium.

The serous type of gland is always affected before the mucous type in the tongue, and advanced destruction of the former is always present before the latter shows definite abnormalities. The submaxillary gland is affected after the tongue has developed outspoken changes. In the submaxillary gland the same sequence of events is noted, namely, dilatation and epithelial metaplasia of the main ducts, accompanied by infection. In both the tongue and submaxillary glands it is frequently noted that only slight metaplastic changes are present while the glands are diffusely infiltrated with polymorphonuclear leukocytes. In every case the dilated ducts are filled with these cells.

Changes in the trachea, bronchi and renal pelvis follow those in the tongue and submaxillary gland. Alterations in the trachea and bronchi are inconstant. The first change is an atrophy of the surface epithelium. This is followed by a dilatation of the ducts of the glands which are filled with polymorphonuclear leukocytes. Changes in the epithelium of the renal pelvis are sometimes observed before those in the respiratory tract. The earliest alterations consist of a

TABLE I

Group I — Basal Diet

Number of rat	Initial weight	Maximum weight	Final weight	Days on diet	Eyes at death	Histological Findings				
						Tongue	Submaxillary gland	Trachea	Bronchi	Renal pelvis
	gm.	gm.	gm.							
12.....	46	150	150	47	Normal	Normal	Normal	Normal	Normal	Normal
20.....	36	112	110	45	Normal	Early metaplasia Infection	Normal	Normal	Normal	Normal
21.....	38	92	86	45	Watery discharge for one day	Early metaplasia Infection	Normal	Normal	Normal	Normal
10.....	46	132	124	51	Watery discharge for one day	Early metaplasia Infection	Few leukocytes in ducts	Atrophy of epithelium	Normal	Normal
23.....	32	102	92	49	Discharge for six days	Marked metaplasia and infection	Duct metaplasia and infection	Normal	Normal	Metaplasia Infection

piling up of the epithelium and an infiltration of polymorphonuclear leukocytes in the subepithelial tissues.

As the disease advances the ducts of the lingual glands dilate more and more, and the infection progresses so that in animals which die of vitamin A deficiency (Table II) the serous glands of the tongue are entirely destroyed by pressure and by infection, with the result that all that can be seen are large abscesses lined by squamous epithelium and filled with polymorphonuclear leukocytes, keratin and necrotic débris. In all advanced cases the epithelial lining of the abscesses is deficient at several points and the surrounding muscle is heavily infiltrated with polymorphonuclear leukocytes and shows extensive necrosis. Gram stains show the abscesses to contain large masses of bacteria in which Gram-positive cocci predominate. In these late cases the submaxillary gland is nearly always involved though occasionally no change other than atrophy is noted. The most frequent finding is large epithelium-lined spaces filled with polymorphonuclear leukocytes while the rest of the gland is diffusely infiltrated with the cells. It is a picture similar to that in the tongue. In a few instances the entire submaxillary gland is converted into an abscess.

The late manifestations in the trachea and bronchi are not uniform as the changes vary all the way from simple atrophy to metaplasia of the surface epithelium and cyst formation in the glands with extensive infection of the surrounding tissue. The epithelium of the renal pelvis is always involved in the late cases. Here the epithelial layer is deeper, keratinization is marked and in some instances the entire renal pelvis is filled with keratin and leukocytes. In several cases calculi blocked the urinary outflow in either the bladder or ureter with the development of pyoureter and pyonephrosis. Two instances of perinephritic abscess developed following rupture of obstructed ureters. The tendency toward hyperplastic activity of the epithelium which has undergone transformation to the squamous type is noted by Wolbach and Howe (1925).[6] This is sometimes very striking and occurs with particular frequency in the tongue and renal pelvis. In the tongue the circle of epithelium surrounding one of the abscesses may be very broad and the outer layers often contain mitotic figures. Occasionally long, finger-like epithelial processes extend into the surrounding tissue and with distortion due to infection the picture may be one difficult to distinguish from malignancy.

TABLE II

Group II — Basal Diet

Number of rat	Initial weight	Maximum weight	Final weight	Days on diet	Eyes at death	Histological Findings				
						Tongue	Submaxillary gland	Trachea	Bronchi	Renal pelvis
	gm.	gm.	gm.							
14.....	35	102	74	42	Xerophthalmia	Metaplasia Abscess	Normal	Atrophy of epithelium	Atrophy of epithelium	Metaplasia Pyonephritis
2.....	36	107	94	48	Xerophthalmia Hypopyon	Metaplasia Abscess	Slight metaplasia. Slight infection	Metaplasia Infection	Metaplasia Infection	Slight metaplasia Infection
8.....	44	88	72	56	Xerophthalmia	Metaplasia Abscess	Atrophy	Normal	Normal	Early metaplasia Infection
3.....	38	96	82	57	Xerophthalmia Hypopyon	Metaplasia Abscess	Slight metaplasia Infection	Atrophy of epithelium	Atrophy of epithelium	Metaplasia Infection
6.....	45	110	82	61	Xerophthalmia	Metaplasia Abscess	Early metaplasia Infection	Atrophy of epithelium	Normal	Metaplasia Infection
27.....	32	92	68	63	Xerophthalmia	Metaplasia Abscess	Metaplasia Abscess	Atrophy of epithelium	Normal	Metaplasia Pyonephrosis
4.....	45	126	80	75	Xerophthalmia Corneal ulceration	Metaplasia Abscess	Metaplasia Abscess	Atrophy of epithelium	Atrophy of epithelium	Metaplasia Infection

TABLE III

Group III — Basal Diet + Cod Liver Oil after Ophthalmia and Loss in Weight

Number of rat	Initial weight	Maximum weight before oil	Weight when oil given	Final weight	Days on oil	Eyes at death	Histological Findings				
							Tongue	Submaxillary gland	Trachea	Bronchi	Renal pelvis
	gm.	*gm.*	*gm.*	*gm.*							
17....	33	120	106	112	4	Normal	Metaplasia Abscess Fibrosis regeneration	Metaplasia of ducts. Infection	Normal	Normal	Hyperplasia Infection
25....	30	56	56	90	14	Normal	Metaplasia Small abscess Fibrosis	Normal	Normal	Normal	Hyperplasia Infection
18....	36	116	98	152	5	Xeroph-thalmia	Metaplasia Abscess	Abscess	Normal	Normal	Metaplasia Pyonephrosis Perinephritic abscess
13....	49	112	112	164	56	Normal	Normal	Normal	Slight infection	Peribronchial fibrosis	Infection
7....	44	124	88	180	91	Normal	Slight meta-plasia Chronic infection	Normal	Normal	Peribronchial fibrosis	Pyonephrosis Perinephritic abscess

In the renal pelvis the hyperplastic changes always overshadow the keratinization. The lining epithelium becomes piled up into a broad layer and columns or sheets of epithelial cells extend into the subepithelial tissue.

After the administration of cod liver oil to rats which have developed xerophthalmia (Table III) the healing process depends upon the extent of destruction of the particular portion by the infectious

TABLE IV.

Group IV — Basal Diet + Cod Liver Oil from Beginning

Number of rat	Initial weight	Maximum weight	Final weight	Days on diet	Lesions
	gm.	*gm.*	*gm.*	*gm.*	
24.....	30	144	144	47	None
22.....	30	110	110	49	None
19.....	33	152	152	49	None
9.....	47	166	166	51	None
26.....	30	140	140	65	None
1.....	47	212	212	73	Bronchitis; bronchopneumonia
15.....	38	152	126	100	None
5.....	46	170	170	106	None
11.....	40	214	200	144	None

process. In the tongue there may be almost complete regeneration of the glandular epithelium with only a few scarred areas remaining. The submaxillary gland often shows no evidence of previous damage. The trachea and bronchi may appear perfectly normal though there is usually some fibrosis of the surrounding tissue. The epithelium of the renal pelvis has shown evidence of hyperplasia with keratinization and infection as long as ninety-one days after the animal has gained weight and is apparently well. The usual picture for as much as two weeks after correction of the diet is the persistence of infection and abnormal epithelium in the tongue and kidney and apparently normal epithelium in the trachea and bronchi. At this

stage there is usually extensive fibroblastic activity and round cell infiltration surrounding the tongue abscesses with evidence of epithelial regeneration in less severely affected portions of the gland. In one animal the only evidence of previous damage to the tongue is a collection of small round cells and endothelial leukocytes replacing part of one of the lobules of the lingual gland.

The control animals (Table IV) were all well nourished at the time of autopsy. The sections from one rat show slight bronchitis and bronchopneumonia. No other abnormal findings were observed in any of the rats in this group.

The tissue changes in the various structures studied have been correlated with age and weight changes and are summarized in the four tables.

Summary

The principal changes associated with vitamin A deficiency in rats are a metaplasia of cuboidal or columnar epithelium in certain parts of the body, epithelial hyperplasia in various structures and infection.

The metaplastic changes involve the following structures in order: the sublingual glands, the submaxillary glands, the epithelium of the renal pelvis and of the trachea and bronchi. The tongue is regularly involved before xerophthalmia appears. The serous type of sublingual gland is the first to be affected. The lesion in the tongue and submaxillary gland begins with a dilatation of the ducts and a metaplasia of the lining epithelium accompanied by infection. In late cases the glandular tissue may be entirely destroyed by pressure from the dilated ducts and by necrosis due to infection. The submaxillary gland is not involved as constantly as the tongue. The epithelium of the renal pelvis may be involved quite early. Metaplasia and infection are always present in the advanced cases. Renal calculi are prone to occur and when obstruction to the urinary outflow is present pyonephrosis develops which is sometimes followed by perinephritic abscess. Epithelial metaplasia of the trachea and bronchi is not common. The most usual finding is an atrophy of the lining cells.

Epithelial hyperplasia is striking in the tongue and renal pelvis. In the latter the hyperplasia overshadows the keratinizing process.

Infection is always present even in the earliest stages and in late cases dominates the picture. No metaplastic activity has been seen without an accompanying infection, but infection has been observed in parts where metaplasia is absent.

If the results of the dietary deficiency are not too severe, xerophthalmia clears rapidly with the administration of cod liver oil and the weight curve rises abruptly. The extent of healing in the various organs depends largely upon the amount of destruction due to infection which is present. Following cod liver oil administration abnormal epithelium and chronic or acute infection persist in the tongue and renal pelvis when the rat is apparently healthy.

REFERENCES

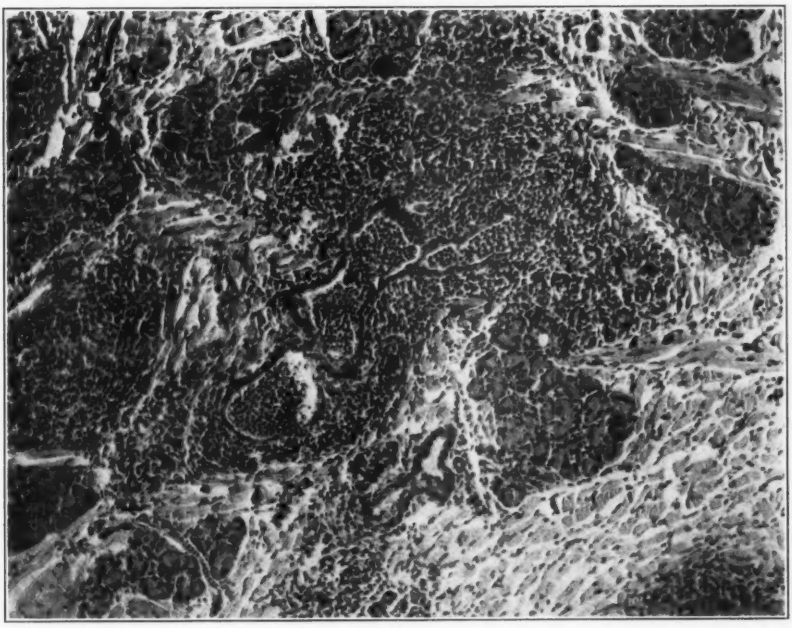
1. Osborne, T. B., and Mendel, L. B. *J. Biol. Chem.*, 1913, xvi, 423.
2. Mori, S. *Bull. Johns Hopkins Hosp.*, 1922, xxxiii, 357.
3. Yudkin, A. M., and Lambert, R. A. *J. Exper. Med.*, 1923, xxxviii, 17.
4. Lambert, R. A., and Yudkin, A. M. *J. Exper. Med.*, 1923, xxxviii, 25.
5. Wason, I. M. *J. A. M. A.*, 1921, lxxvi, 908.
6. Wolbach, S. B., and Howe, P. R. *J. Exper. Med.*, 1925, xlii, 753.
7. Goldblatt, H., and Benischek, M. *J. Exper. Med.*, 1927, xlvi, 699.
8. Sherman, H. C., and Munsell, H. E. *J. Am. Chem. Soc.*, 1925, xlvii, 1639.
9. Osborne, T. B., and Mendel, L. B. *J. A. M. A.*, 1917, lxix, 32.
10. Osborne, T. B., and Mendel, L. B. *J. Biol. Chem.*, 1919, xxxvii, 557.
11. Van Leersum, E. C. *J. Biol. Chem.*, 1928, lxxvi, 137.
12. Hess, A. F. *J. A. M. A.*, 1927, lxxxix, 337.
13. Parsons, H. T. *J. Biol. Chem.*, 1920, xliv, 587.
14. Parsons, H. T., and Hutton, M. K. *J. Biol. Chem.*, 1924, lix, 97.
15. Lepkovsky, S., and Nelson, M. T. *J. Biol. Chem.*, 1924, lix, 91.

DESCRIPTION OF PLATES

PLATE 17

FIG 1. Rat 21. Serous portion of sublingual gland. Early lesion. Dilatation of ducts with metaplasia of the lining epithelium. Polymorphonuclear leukocytic exudate in ducts and adjacent glandular tissue. ×125.

FIG. 2. Rat 6. Cross-section of tongue. Advanced lesion. Great dilatation of ducts with epithelial metaplasia. Exudate in ducts and surrounding muscle. Necrosis of muscle and supporting tissue. Hyperplasia of the surface epithelium. Drawing ×1150.



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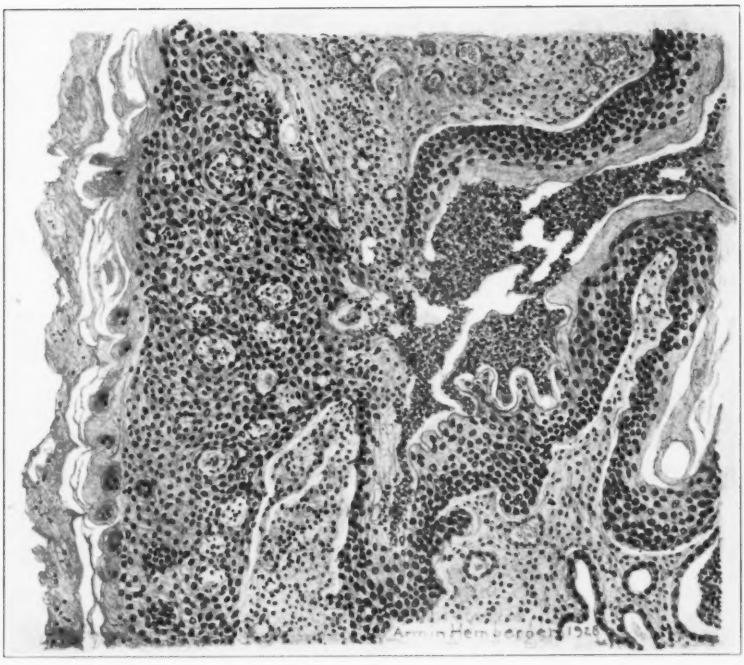


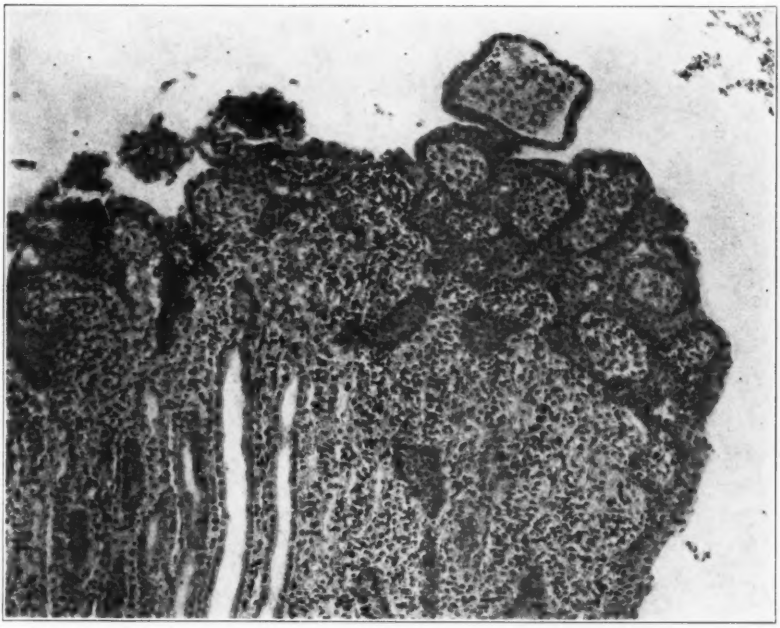
2

Tyson and Smith Vitamin A Deficiency in Rat

Plate 18

FIG. 3. Rat 25. Hyperplasia of epithelium of renal pelvis. Exudate in epithelial layer and subepithelial tissues. ×125.

FIG. 4. Rat 25. Abscess of tongue partly lined by metaplastic epithelium. Keratosis not marked. Extensive fibroblastic activity surrounding the abscess. ×65.



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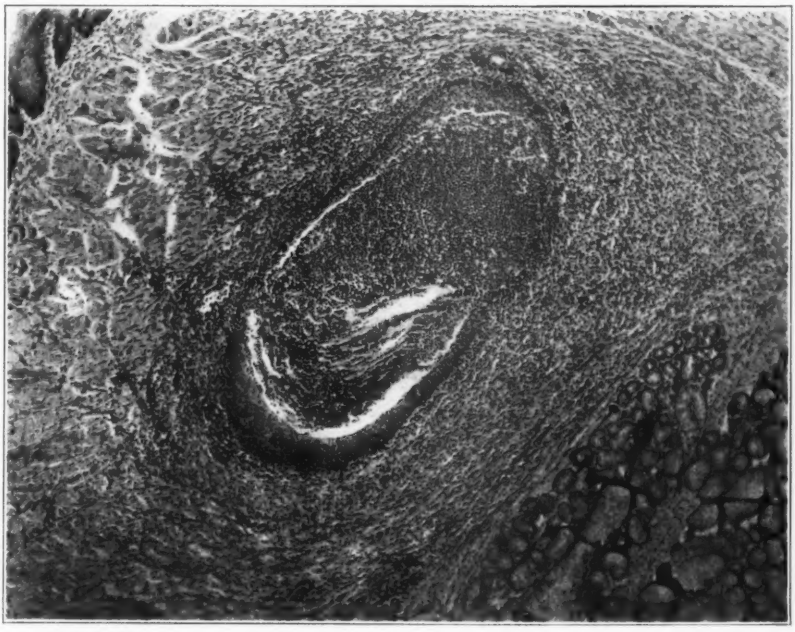

4

Tyson and Smith Vitamin A Deficiency in Rat

STUDIES ON COMPENSATORY HYPERTROPHY OF THE THYROID GLAND*

VIII. A COMPARISON BETWEEN THE EFFECT OF ADMINISTRATION OF THYROXIN, THYROID AND ANTERIOR PITUITARY SUBSTANCE ON THE COMPENSATORY HYPERTROPHY OF THE THYROID GLAND IN THE GUINEA PIG

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In a previous investigation [1] we have shown that feeding of guinea pigs with thyroid tablets (Armour & Co.) prevents the compensatory hypertrophy of the thyroid gland. Subsequently in association with E. E. Kaplan [2] we found that feeding with anterior pituitary substance (Armour & Co.) acts in a similar manner to the thyroid substance; it also prevents compensatory hypertrophy. In the present investigation we wished (1) to confirm our previous results concerning the action of anterior pituitary substance, as this seemed desirable in view of the prevalent belief that feeding with this substance exerts no definite action; (2) to compare with the effect of feeding thyroid tablets that following the administration of thyroxin, it being at present a debated question whether or not thyroid substance contains active substances in addition to thyroxin; (3) to determine the relative potency of these various substances as far as their action on the compensatory hypertophy of the thyroid is concerned.

I. THE EFFECT OF FEEDING WITH ANTERIOR PITUITARY SUBSTANCE

In eleven guinea pigs one whole lobe and three-fourths of the second lobe of the thyroid gland were removed. Two days after the operation each animal received by mouth five grains of anterior pituitary substance in tablet form (Armour & Co.) and this dose was continued daily. Thirty to thirty-two days after operation the remaining parts of the gland were removed. The experiments were carried out during January and February. The initial weight of the

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guinea pigs varied between 342 and 446 gm. In seven animals there was a considerable increase in weight during this period; in one animal the weight remained approximately stationary and in three others the weight at the end of the experiment was not recorded. In order to render unnecessary detailed descriptions of our findings we shall make use of a system of grades which we introduced previously[3] for this purpose. In eight guinea pigs the grade indicating the hypertrophy or lack of hypertrophy varied between 5 and 5.25, while in three additional guinea pigs the grade varied between 4.85 and 5. There was therefore no hypertrophy found in these glands, notwithstanding a decided increase in weight during the period of the experiment, in the majority of animals. However, feeding with anterior pituitary substance not only prevented hypertrophy from taking place, but in this group the acini were markedly below the average size, contained rather solid and slightly retracted colloid, epithelium somewhat below medium size and rather infrequent phagocytes which were usually small and unable to dissolve the hard colloid. While these characteristics prevailed on the whole, there were found in some glands areas where the acini were somewhat larger and the acinus cells likewise higher, without, however, becoming definitely hypertrophic. In such acini, also, the colloid was at times somewhat less hard, adhering to the epithelium by means of threads between which vacuoles were included. Occasionally a larger number of phagocytes were found in the colloid of such acini. In some places an intermediate condition existed.

In control animals in which also the greater part of the thyroid gland had been removed, but in which the pituitary extract was not administered, the size of the epithelial cells was higher on the average, the colloid softer and the peripheral vacuolar zones or solution processes in the colloid were seen more frequently. The average size of the acini was likewise greater and the acini showed often an irregular outline in the controls, in contrast with the more regular outline of the usually smaller acini in the glands of the guinea pigs fed with pituitary substance. Whereas in the glands of the latter animals, mitoses, if they occurred at all, were very rare, they were observed much more frequently in the controls and the phagocytes were here on the average larger and more numerous. The breaking through of the walls of neighboring acini and the development of papillae were encountered more often in the controls although they

occurred also in the pituitary-fed guinea pigs. In addition there were some cases among the controls in which the hypertrophy was quite pronounced, whereas this did not occur in the pituitary group. In comparing thus the condition of the thyroid remnants of animals fed with pituitary substance and of controls, we must take into consideration the fact that in this series a majority of the control animals had lost weight, or at least had not gained in weight during the course of the experiment, while a majority of the guinea pigs in the pituitary group had gained in weight, and thus the real difference between the pituitary-fed and the control animals was greater than is indicated in these experiments.

II. The Effect of Feeding Thyroid on Compensatory Hypertrophy of the Thyroid Gland

Eight guinea pigs with an initial weight varying between 315 and 352 gm. were used in this series. Beginning two days after total extirpation of one lobe and three-fourths of the other lobe of the thyroid gland, some of the animals were fed with two to four tablets (Armour & Co.) of 1/10 grain of thyroid substance; in others four tablets were administered during the first week or two, then the dose was reduced to two tablets. In one case six tablets were given during the first thirteen days, when the dose was reduced to three tablets. In general our aim was to administer thyroid substance in such a way that the guinea pigs gained in weight during the course of the experiment. In several guinea pigs therefore the administration was interrupted during the experiment for a few days in order to prevent a loss of weight. As a result of these precautions we succeeded in all the animals except one, which lost 25 gm., in obtaining a gain in weight during the course of the experiment which extended over a period of thirty to thirty-five days.

Notwithstanding this gain in weight the remnants of the thyroid gland showed inactive gland tissue. The acini were mostly of small size and were lined with very flat, or low to medium epithelium. However, in some areas the acini were a little larger. The colloid was usually solid and slightly retracted. Rarely there was a somewhat softer colloid adherent to the epithelium and a peripheral vacuolar zone was observed, or a greater retraction of the colloid had taken place indicating a more marked solution process. The

number of phagocytes contained in the colloid was on the whole very small, especially in acini lined with flat epithelium; where the epithelium was of medium size the phagocytes were often slightly more numerous. Usually they did not succeed in digesting the hard colloid but occasionally they seemed to cut out some balls of it. The grade characterizing such a condition of the thyroid gland lies somewhere between 5 and 6, indicating a tendency to shrink on the part of the acini and of the epithelium and an increasing hardness of the colloid.

In general this reduction in the size of the glandular elements and the corresponding increase in the hardness of the colloid was greater in the series fed with thyroid tablets than in those fed with anterior pituitary substance, notwithstanding the fact that the gain in weight was equally present in both series. It is, therefore, very probable that the administration of thyroid substance is more effective than the anterior pituitary in preventing hypertrophy of the thyroid gland and in reducing its size and function, the former producing the structural signs of functional inactivity of the gland to a still higher degree than the former.

III. The Effect of Administration of Thyroxin

A group of eight guinea pigs were treated in the same way as the two former groups, except that instead of being fed with thyroid tablets they received by mouth tablets containing 1/320 of a grain each of thyroxin. In some cases four to six tablets were administered in the beginning but after 9 to 12 days this dose was reduced to one-half this amount. In one animal as little as one tablet was given daily. In several guinea pigs the administration of thyroxin had to be interrupted for several days because this substance caused a loss of weight, the weight in the beginning of the experiment varying between 321 and 409 gm.; only three of the animals gained in weight during the course of the experiment, while four remained about stationary, and one decreased considerably. The last-mentioned guinea pig died twenty-four days after the removal of the greater part of the thyroid gland, and inasmuch as no remnants of the thyroid gland were found at the time of examination it had to be excluded in considering the results. There thus remained seven guinea pigs in none of which was any hypertrophy noted. In gen-

eral the condition of the remaining parts of the glands was similar to that seen after feeding of thyroid tablets; the average grade was also about the same in both cases ranging somewhere between 5 and 6. The small-sized acini predominated, but there were seen some acini of medium size. The height of the epithelial cells was usually low or it ranged between low and medium. The colloid was generally hard and slightly retracted but occasionally it was somewhat more retracted as a result of a certain degree of softening, or it adhered with threads to the epithelium and showed a vacuolar zone in the periphery. The number of the phagocytes was on the whole very small; only rarely some larger-sized phagocytes were seen in the colloid or occasionally even an acinus was filled with small phagocytes. Collections of lymphocytes were not seen in the stroma.

We find then that thyroxin acts in the same way as thyroid substance when fed to guinea pigs, both preventing hypertrophy and reducing the activity of the thyroid gland to about equal degrees.

Discussion and Conclusions

These investigations confirm our previous conclusions concerning the effect of feeding anterior pituitary gland substance (Armour & Co.) on the thyroid in guinea pigs; it prevents compensatory hypertrophy of this organ and tends to produce in it a condition corresponding to a resting stage. In general it decreases the size of the acini and the acinus epithelium becomes lower; it also causes a hardening of the colloid. In this respect, therefore, administration of anterior pituitary substance resembles in its action feeding with thyroid gland substance; however, it is not quite so effective as the latter. Feeding with anterior pituitary substance does not reduce the average size of the acini and of epithelial cells to the same extent as does thyroid substance. In the former instance there may still be found in various glands or in certain places in a single gland, a greater number of medium-sized acini and epithelial cells; the colloid may at such points be a little softer, a greater size of acini and of epithelial cells being usually associated with a decreased hardness of colloid. In accordance with the reduction in activity indicated by the structural changes resulting from administration of these two substances there is a diminution in the number of phagocytes found, and the phagocytes that do enter the colloid are usually small in size and do not succeed in causing a localized solution of the colloid;

furthermore, the collections of lymphocytes which are occasionally observed in controls and especially in the iodine-fed guinea pigs are absent in these thyroid glands, a further evidence of reduced gland activity. The solution of walls separating neighboring acini, although it does occur, is rare.

We see then that feeding with anterior pituitary substance as well as with thyroid substance tends not only to prevent compensatory hypertrophy in the guinea pig, but also to reduce the activity of the gland below the level of that of a normal gland; in this respect, however, thyroid substance is in all probability somewhat more effective. In addition, our experiments show that thyroxin very much resembles thyroid substance in its action, at least in the prevention of compensatory hypertrophy, as well as in the production of those structural changes which indicate functional inactivity. We may therefore conclude that it is the thyroid hormone itself and not another substance mixed with it which exerts the typical effect on the thyroid gland. As to the ingredient in Armour's preparation of anterior pituitary substance which exerts this effect similar to that of thyroxin, we are not in a position to make a definite statement, except that we know that it cannot be due to the admixture of inorganic iodine because the latter does not depress the activity of the thyroid gland, but on the contrary stimulates it; but whether an organic iodine substance resembling thyroxin in its action is responsible for this effect is a question that must be left open at the present time.

We find thus certain structural features which are correlated and which form a complex indicative of thyroid inactivity. The main features of this condition are: (1) small size and regularity in shape of acini; (2) relative flatness of epithelium; (3) decrease in number of mitoses; (4) hardness of colloid; (5) diminution in number, size and activity of the phagocytes; (6) probable decrease in number of lymphocytes found in these glands. It is of interest to contrast with this combination of features the condition produced by factors causing a stimulation of the gland. Here we find, at least in certain areas: (1) larger than medium-sized and often irregular acini; (2) higher epithelium; (3) increase in number of mitoses; (4) softness of colloid; (5) increase in number, size and activity of phagocytes; (6) a probable increase in accumulation of lymphocytes and in number of spurs and papillae reaching into the cavity of the acini. Compen-

satory hypertrophy and other types of hypertrophy lead to this latter condition; at least certain of these features are produced also by the administration of potassium iodide until such a time as secondary changes take place.

The similarity in the action of thyroid hormone and of anterior pituitary substance which we thus find, is in harmony with some other facts which likewise point to a relationship between these two substances. It is known that removal of the thyroid gland sets in motion certain hypertrophic processes in the anterior lobe of the pituitary gland. Thyroid substance and thyroxin prevent compensatory hypertrophy and change the structure of an active into that of an inactive gland by supplying that constituent, the lack of which sets into motion hypertrophic changes; and we must assume that the anterior pituitary also supplies a certain constituent which in some respects acts similarly to that given off by the thyroid gland, preventing compensatory hypertrophy and inducing structural changes which lead to cessation of gland functions. Thus both functional activity and structural features are coördinated in the effect of thyroid, anterior pituitary substance and thyroxin administration; after KI such a coördination also exists but in this case it produces results of an opposite character.

It is interesting to refer briefly in this connection to the comparative effects of thyroid and anterior pituitary substance on the metamorphosis of amphibian larvae. It is well known that feeding thyroid substance accelerates this process. Now, Hogben [4] found that injection of anterior pituitary substance (Armour & Co.) also induces metamorphosis in the Mexican axolotol. Smith [5] working with the Colorado axolotol likewise obtained positive results in feeding this preparation, but believed this effect to have been caused by an admixture of thyroid gland to Armour's anterior pituitary substance. However, Spaul [6] as well as Uhlenhuth and Schwartzbach [7] attributed the metamorphosis induced by injection of anterior pituitary substance to the pituitary preparation as such; in particular Uhlenhuth and Schwartzbach could show that the metamorphosis in this case depends in all probability upon a stimulating effect which the injected material exerts on the thyroid gland. It is this marked stimulation of the thyroid gland in the axolotol which distinguishes sharply the effect of anterior pituitary in the axolotol and in the guinea pig; in the latter animal we have shown that this substance

causes a depression in the thyroid gland; therefore its action on the guinea pig is the reverse of the action in amphibia as observed by Uhlenhuth and Schwartzbach. The results obtained by the last-named authors concerning the stimulative effect of anterior pituitary substance on the thyroid gland are in agreement with some observations of Allen.[8] We must therefore consider the possibility that the action of anterior pituitary substance on the thyroid gland in mammals and in amphibia may be different in kind, or that in both cases different constituents of the anterior pituitary glands are active.

Summary

1. The inhibiting effect of feeding anterior pituitary substance on compensatory hypertrophy of the thyroid gland previously found by Loeb and Kaplan is confirmed.

2. Anterior pituitary substance, thyroid substance and thyroxin all produce similar effects, not only preventing compensatory hypertrophy of the thyroid gland but also tending to produce changes in the gland that signify a resting condition.

3. There probably exist quantitative differences between the effectiveness of these substances, anterior pituitary extract being somewhat less effective than the others.

4. This similarity in the effects of thyroid and anterior pituitary substances on the thyroid gland is in accordance with certain other similarities which have been found to exist between these substances, but is not in harmony with the action of anterior pituitary observed in larvae of Urodele amphibians. It is suggested that different substances may perhaps be responsible for the effects exerted by administration of anterior pituitary substance under different conditions.

REFERENCES

1. Loeb, Leo. *J. Med. Res.*, 1920, xlii, 77.
2. Loeb, Leo, and Kaplan, E. E. *J. Med. Res.*, 1924, xliv, 557.
3. Loeb, Leo. *Am. J. Path.*, 1926, ii, 19.
4. Hogben, Lancelot. *Proc. Roy. Soc. Med.*, 1923, xciv, 204.
5. Smith, P. E. *Brit. J. Exper. Biol.*, 1926, iii, 239.
6. Spaul, E. A. *Brit. J. Exper. Biol.*, 1925, ii, 427.
7. Uhlenhuth, E., and Schwartzbach, S. *Brit. J. Exper. Biol.*, 1927, v, 1.
8. Allen, B. F. *University of California Publications in Zoölogy*, 1927, xxxi, 53.

STUDIES ON COMPENSATORY HYPERTROPHY OF THE THYROID GLAND *

IX. THE INFLUENCE OF VARIATIONS IN SIZE OF THE REMAINING PART OF THE GLAND, IN MODE OF ADMINISTRATION AND IN QUANTITY OF POTASSIUM IODIDE ON THE HYPERTROPHY OF THE THYROID IN THE GUINEA PIG

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In former communications we have shown that in guinea pigs in which the greater part of the thyroid gland had been removed, so that only about one-third to one-fifth of one lobe was left, the feeding of potassium iodide during a period of four to five weeks following the operation not only did not prevent the establishment of compensatory hypertrophy in the remaining gland tissue but, on the contrary, tended to increase the number of cases in which hypertrophy occurred and furthermore to intensify in general the degree of the hypertrophy.[1, 2] The view has been expressed by Marine [3] that if as much as one-half of one lobe is left, instead of a smaller part, administration of potassium iodide prevents hypertrophy. In our new series, therefore, as much as one-half of one lobe of thyroid remained in the animal. In addition we thought it of interest to determine the effect of variations in the amount of KI given following the operation. For this purpose we administered the same quantities of this substance which subsequently Rabinovitch used in order to test the effect of variations in the dose of KI given on the intensity of mitotic cell proliferation in the thyroid of the guinea pig. Rabinovitch found a definite relationship between the amount of iodine administered to the animals daily and the resulting cell multiplication. Furthermore, we wished to determine whether intraperitoneal injection of KI has the same effect as feeding of this substance by mouth.

We carried out two series of experiments; in both the examination took place usually thirty to thirty-two days following the operation, except in a few experiments of the second series in which it occurred

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as early as twenty days following extirpation of the gland. In the first series, carried out in the spring of 1926, the iodide was fed by mouth, whereas in the second series, carried out in the early part of 1928, intraperitoneal injections were given daily to the guinea pigs. In both series some of the animals received 0.01, others 0.05 and a third group as much as 0.1 gm. KI daily, beginning one or two days following the operation. In addition there was in each series a control group of guinea pigs which were treated in the same way as the other animals except that KI was not administered following the extirpation of the gland. In describing the results obtained we shall make use of the system of grades introduced by us in a former paper.[2] In doing so we must, however, take cognizance of the fact that the iodide induces certain changes in the gland tissue of the hypertrophying gland which are not different in principle, but which differ from those taking place in the controls as far as their intensity is concerned. Prominent among these changes is a tendency to a more rapid and complete softening and liquefaction of the colloid in the iodized guinea pigs. Furthermore, there is in many acini a delay in absorption of this liquefied material and thus subsequently a distension of the acini associated with secondary pressure effects may take place in the gland. In estimating the relative degree of compensatory hypertrophy in the iodized and control guinea pigs, we must take this factor into consideration.

Series I. KI Fed to Guinea Pigs in Form of Pills

Control Animals: Ten guinea pigs were used as controls; their initial weight varied between 413 and 540 gm., with one exception in which the weight was 325 gm. The grades of hypertrophy were: 4.50; 3.80; 4.90; 4.25; 4.90; 5.50; 3.85; 4.40; 5.0. One of the animals died prematurely; it had the grade 5.0. The average degree of hypertrophy was therefore relatively low, 4.70. We have thus far found two variable factors which influence the degree of hypertrophy in guinea pigs in which an equal amount of thyroid gland has been removed: namely, (1) the season of the year in which the experiment is carried out — during the summer months the hypertrophy is on the average less than during the rest of the year; (2) a gain in weight during the course of the experiment; animals which gain have a higher average of hypertrophy than those which do not gain weight. There are probably still other factors involved which need further

analysis. In this case the failure of the animals to gain weight during the experiment seems largely to account for the low degree of hypertrophy obtained. Of the ten animals, only three were heavier at the end of the experiment than in the beginning and two of these showed the highest grades; one, which gained only a few grams, is included in the stationary class, comprising three guinea pigs. Five animals showed a decided loss of weight during the experiment; they showed very little or no hypertrophy. As we shall see later the hypertrophy was much more pronounced in the control animals of the second series, which showed a much greater gain in weight during the experiment than the animals of the first series.

Guinea Pigs Fed with 0.01 gm. KI Daily: Eight guinea pigs were used in this group. The average grade of hypertrophy was 4.0, and the individual grades were 4.0; 3.50; 4.50; 4.25; 4.50; 3.50; 4.50; 3.25. The degree of hypertrophy is therefore decidedly higher here than in the controls. We cannot attribute the differences in hypertrophy found in this series entirely to the KI action, inasmuch as in this group six guinea pigs showed a moderate gain in weight, amounting on the average to about 20 to 40 gm. while of the two remaining animals one lost weight and the second remained about stationary. However, the structural features of KI feeding were clearly present in these animals and we may attribute, in part at least, their increase in hypertrophy over that of the controls to the administration of KI.

Guinea Pigs Fed with 0.05 gm. KI Daily: Nine guinea pigs, the initial weight of which ranged between 470 and 538 gm., were used in this group. All of these animals, except two, gained in weight during the course of the experiment. In the two which lost weight the hypertrophy was very slight, the grades being 4.90 and 4.50 respectively; in the others the grades were as follows: 4.0; 3.0; 3.0; 3.25; 4.0; 3.25; 4.75. The average grade in this group was 3.85. Again the hypertrophy was distinctly higher than in the controls and even than in the preceding group of iodized animals.

Guinea Pigs Fed with 0.1 gm. KI Daily: Eight guinea pigs, with an initial weight varying between 450 and 560 gm. served for these experiments. Three of these animals lost weight; the remaining five showed a stationary weight or a very slight gain. The average grade in this group was 4.55; the grades of the animals which lost weight were on the average lower than those of the others.

There is reason for assuming that the low degree of hypertrophy in this group is at least partly due to the unfavorable weight balance in these guinea pigs; but it is also possible that such a large dose of KI, corresponding approximately to a daily dose of 12 to 15 gm. of this substance in an adult man, has either, in some unknown way, an injurious effect upon the organism, or that it causes an excess thyroxin formation leading to a loss in weight and indirectly diminishing the activity of the thyroid gland, thus causing the diminution in hypertrophy observed.

However, when in this group hypertrophy is present the characteristic features of the latter do not differ from those usually observed, but on the average less hypertrophy occurred. The acini were smaller and the colloid more solid; yet the phagocytes were increased in number even in places in which only a trace of hypertrophy could be found, although they were not increased where hypertrophy was completely lacking. As a result of the increased solution processes in the colloid, pressure effects and occasional perforations of the walls separating adjoining acini, due to increased pressure, were observed. Here again the solution of the colloid took place by means of an agent, presumably of an enzymatic nature, acting diffusely as well as by means of phagocytes acting locally.

Series II. KI Injected Intraperitoneally

In this series the same quantities of KI, which in the first series were fed to guinea pigs, were injected intraperitoneally.

Control Animals: The initial weight in this group varied between 305 and 405 gm. except in two guinea pigs in which it was somewhat lower. Two animals examined after twenty days showed definite hypertrophy with the grades 3.0 and 3.20 and with structural changes characteristic of compensatory hypertrophy; both of these guinea pigs gained 40 to 50 gm. in weight during the course of the experiment.

Four guinea pigs were examined after thirty days, but one of these must be excluded from the list because it showed, evidently as the result of a local infection, infiltration of the gland tissue and the surrounding capsule with many polymorphonuclear leucocytes and with lymphocytes. The colloid in this case was largely liquefied and therefore increased in volume; thus pressure was exerted on the walls of the acini, but notwithstanding these effects there were still

many mitoses as well as other signs of hypertrophy observed. The grades of the two other guinea pigs, that had gained considerably in weight, were 2.80 and 3.75, whereas in an animal with almost stationary weight the hypertrophy was almost lacking, the grade being 4.75.

We may refer here to three additional control experiments carried out recently in which only one-third of one lobe had been left behind, and in which the examination took place thirty days following operation. The initial weight of these animals was between 400 and 500 gm. and they gained considerably in weight during the course of the experiment. They showed moderate degrees of hypertrophy, their grades varying between 3.50 and 3.90. While, as usual, the changes in these gland remnants were in principle similar to those observed in the iodized guinea pigs, still the typical quantitative differences, to which we have referred, were again noticeable; in particular, the marked variegation, characteristic of the KI animals, was here lacking and the colloid showed, on the whole, much less pronounced solution processes, although in individual acini the same conditions could be found as in the iodized guinea pigs.

Guinea Pigs Injected Daily with 0.01 gm. KI: The initial weight of the four animals in this group, two of which were examined after twenty days and two after thirty days, varied between 333 and 438 gm. Three of these guinea pigs gained in weight and only one, examined after thirty days, remained stationary. All the animals showed distinctly hypertrophic thyroid remnants; after twenty days the grades were 3.40 and 3.50 and after thirty days the grade was 3.0 in both animals. Lack of gain in weight thus did not prevent hypertrophy. The effects of iodine were typical in the thyroid remnants of these animals: they consisted of softening and solution of colloid, hypertrophy of epithelium with secondary flattening as the result of pressure, perforation of walls (except in a case where the connective tissue between the acini was increased and thus prevented perforation) and in general there was found the characteristic variegated appearance of the thyroid gland. It is of interest to note that these conditions were present after twenty, as well as after thirty days.

Guinea Pigs Injected Daily with 0.05 gm. KI: Of the four animals used in this group, two were examined after twenty, and two after thirty days; the initial weight of these guinea pigs varied between

308 and 420 gm. and all of them gained in weight during the course of the experiment. The gland remnants showed in each case distinct hypertrophy, the grades after twenty days being approximately 2.75 and after thirty days, 2.0 and 2.75.

The histological picture was characteristic of KI administration, after twenty, as well as after thirty days following operation. One case in particular, examined after thirty days, showed a very pronounced hypertrophy in that the whole center of the gland remnant had been converted into a system of irregular slits lined with very hypertrophic epithelium; the greater part of the colloid had been absorbed in these acini.

Guinea Pigs Injected Daily with 0.1 gm. KI: Of the four animals in this group, in two the examination took place after twenty days and in the other two after thirty and thirty-one days, respectively, following operation. The initial weight in these guinea pigs varied between 320 and 435 gm. In the animal examined after twenty days, which had lost in weight, the grade was 4.90, indicating that there was at best only a trace of hypertrophy. In the second guinea pig, which had remained stationary in weight, the grade was 3.90. The epithelium was hypertrophic; the colloid slightly softened and adherent to the epithelium with long threads. There were some groups of acini with pale colloid and hypertrophic epithelium, and on the other hand, some peripheral non-hypertrophic acini with hard colloid. In some acini the colloid had been eaten up by phagocytes. In the animal examined after thirty-one days, the weight had remained stationary and the grade was 3.50, while in the third guinea pig, with an increase in weight after thirty days, the grade was 4.0. These animals gained on the average less in weight than the others in this series, and correspondingly the average of hypertrophy was less. However, in the individual cases there was no definite parallelism between change in weight and degree of hypertrophy.

It is of special interest that even so large a dose as 0.1 gm. KI given daily does not necessarily prevent hypertrophy. It is possible that the very large dose is less favorable to the development of compensatory hypertrophy than the smaller doses; under similar conditions we found in the preceding series, in which KI was fed to the guinea pigs instead of being injected, the hypertrophy was less marked than in the animals receiving 0.01 and 0.05 gm. KI daily. Again the possibility exists that in these animals an excess of thy-

roxin was produced which counteracted to some extent the production of hypertrophy. However, the number of our experiments is as yet too small to allow a definite conclusion in this respect.

The structural characteristics in these gland remnants were similar to those found in the other groups of iodized guinea pigs, except that the proportion of acini which were smaller, with fewer phagocytes, a relatively lower epithelium and a harder colloid, was greater in this group.

If we compare the average degrees of hypertrophy in the control and iodized animals of the second series, we find the following figures: in control guinea pigs, 3.50; in guinea pigs injected with 0.01 or 0.05 gm. KI, 2.90; in guinea pigs injected with 0.1 gm. KI, 4.0.

The average degree of hypertrophy is, therefore, somewhat higher in the animals injected with 0.01 and 0.05 gm. KI daily than in the control animals, whereas a daily injection of 0.1 gm. KI was less favorable for the development of hypertrophy, although it did not prevent it.

Summary and Conclusions

1. Our new investigations concerning the action of KI in compensatory hypertrophy of the thyroid gland in the guinea pig confirm our previous conclusions, that administration of KI does not prevent hypertrophy, but that, on the contrary, the average degree of hypertrophy may be even higher in the animals to which as large a dose as 0.05 gm. KI has been administered daily than in the controls. In estimating the intensity of hypertrophy we must, however, take into consideration two facts which complicate the comparative rating of hypertrophy in iodized and control animals. (1) The change in weight of the animals during the course of hypertrophy is one of the variable factors which may affect the intensity of hypertrophy, and (2) secondary pressure effects, especially in iodized animals, may obscure the hypertrophic changes which had been previously established. Furthermore, removal of a great part of the thyroid gland as such acts as a stimulus to the gland, and the administration of iodine acting in this case as a superimposed stimulus, may be less effective than the administration of iodine to normal guinea pigs. Yet, notwithstanding these complicating factors, a consideration of all our series combined, in which iodine was administered to guinea pigs during the course of compensatory hypertrophy, makes it at least very probable that iodine itensifies rather than diminishes compensatory hypertrophy.

2. Our conclusions concerning the effects of iodine on compensatory hypertrophy hold good whether we leave behind only one-third to one-fifth of one lobe of thyroid gland as was done in our previous experiments, or one-half of one lobe as in our present experiments. Furthermore, we found that the effects are in principle the same, whether the iodine is fed by mouth or injected intraperitoneally.

3. An increase of the daily dose of KI to 0.1 gm. per day, corresponding to about 13 to 15 gm. of this substance in an adult person, although it did not prevent the establishment of compensatory hypertrophy, tended in our two series of experiments to diminish its intensity. Whether this result was due to a greater average loss in weight, produced through a non-specific action of so large an amount of KI in these animals, or whether conversely the loss of weight was due to an excess activity of the thyroid remnant called forth by so large a dose of KI, and an inhibiting effect of thyroxin, thus formed, on compensatory hypertrophy, must be left undecided at the present time.

4. The greater variegation in the structure of groups of acini in the KI fed animals, as compared with the controls, makes it in many cases possible to distinguish the compensatory hypertrophy of the thyroid gland, as it is found in the former, from the corresponding condition in the latter group of animals.

REFERENCES

1. Loeb, Leo. *J. Med. Res.*, 1920, xli, 481.
2. Loeb, Leo. *Am. J. Path.*, 1926, ii, 19.
3. Marine, David. *Arch. Path. & Lab. Med.*, 1926, ii, 829.

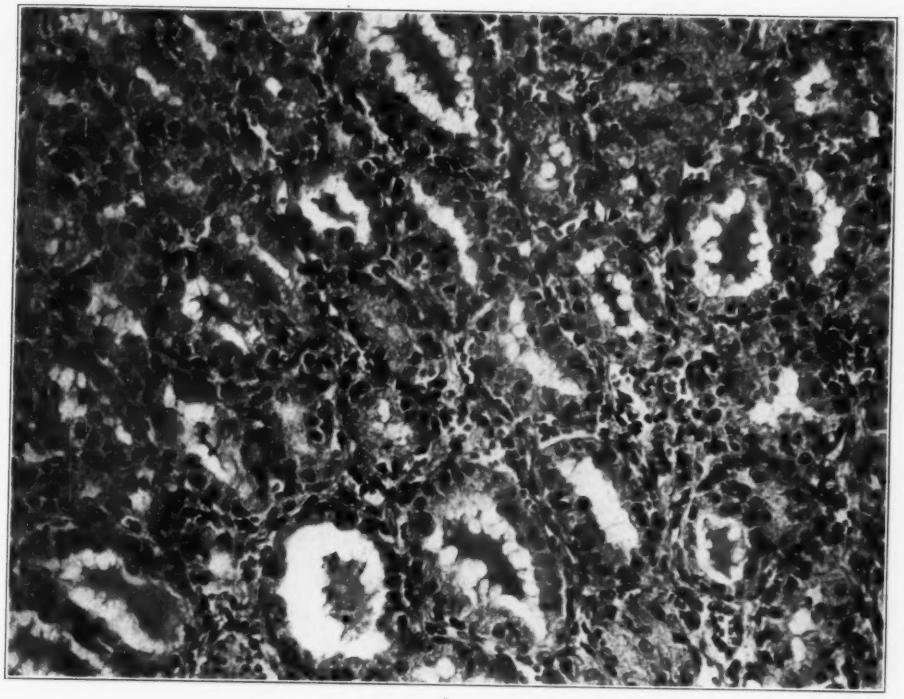
DESCRIPTION OF PLATE

PLATE 19

FIG. 1 and Fig. 2 are from Guinea Pig 945, male, weight 308 gm.; 1½ lobes of thyroid were extirpated on April 12, 1928; following the operation daily intraperitoneal injections of 0.05 gm. KI were given. Remaining part of thyroid taken out thirty days later, May 12, 1928; weight of guinea pig 365 gm.

FIG. 1 shows a part from the center of the remnant; there is marked hypertrophy of the acinus cells; the colloid is much diminished or lacking altogether. A number of the acini have been converted into slits. A mitosis is seen near the upper margin of the picture. × 300.

FIG. 2 shows a higher magnification of another part of the same piece. The colloid has entirely disappeared; the acinus cells are high. A mitosis is seen in the center of the picture. In the periphery of this piece the acini were much larger and distended by colloid which was in process of liquefaction. × 580.



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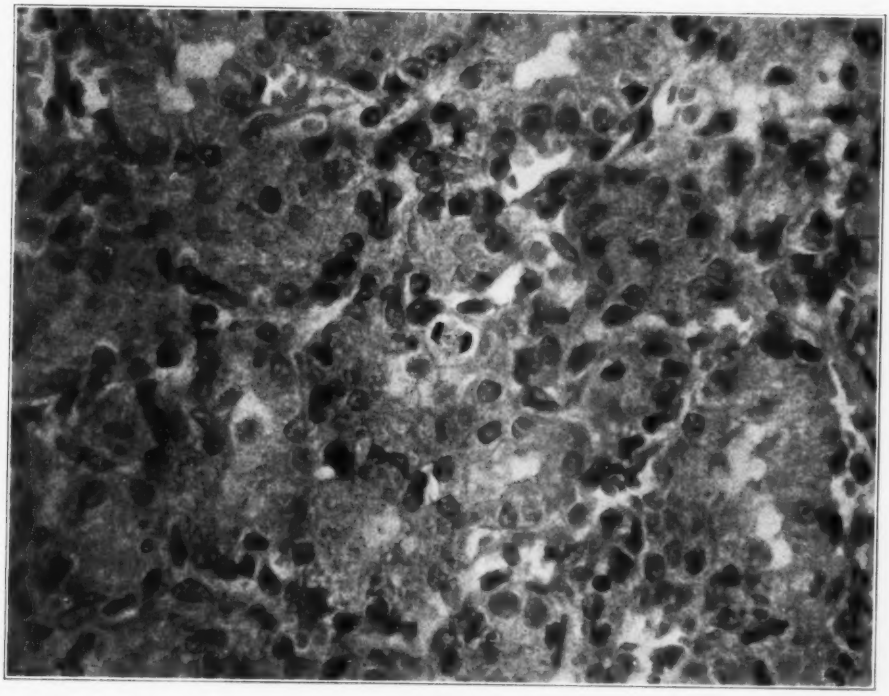

2

Loeb Compensatory Hypertrophy of Thyroid Gland

THE EFFECT OF UNDERFEEDING ON THE PROLIFERATIVE ACTIVITY OF THE THYROID GLAND IN THE GUINEA PIG *

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In previous experiments Loeb [1] has observed that the degree of compensatory hypertrophy in the thyroid glands of guinea pigs is influenced by the changes in weight which the animals undergo during the period following partial removal of the glands. On the average the hypertophy was more marked in guinea pigs showing an increase in weight as compared with those in which the weight remained stationary or decreased, although in individual cases a loss in weight did not necessarily prevent hypertrophy. The change in weight constituted thus one of the variable factors which determined the growth processes in the thyroid gland. In accordance with these observations I found subsequently that the proliferation which occurs in the thyroid gland of normal guinea pigs under the influence of potassium iodide is on the average greater in animals which gain in weight during the course of the experiment than in those in which a decrease in weight takes place. These findings, together with other observations previously made in this laboratory [2] concerning the effect of undernourishment on the growth of the ovarian follicle and of the epidermis (to be published later), suggested the following investigation into the influence of undernourishment on the proliferative activity of the normal thyroid gland in guinea pigs. In regard to previous observations concerning the effect of inanition on the thyroid gland we may refer to the work of Jackson,[3, 4] and of Morgulis [5] in which the literature is fully discussed. Jackson made a very careful study of the thyroid gland in rats. In his experiments the period of underfeeding was much longer than in our investigations; he observed therefore much further advanced stages, especially marked degenerative processes. These are lacking in the guinea pigs studied by us. Furthermore our investigations differ from earlier ones inasmuch as we carried out a quantitative comparison of the

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proliferative activity in the underfed animals with the proliferative activity in the normal thyroid gland.

For this study twelve male guinea pigs of approximately the same weight were selected; five of them were underfed during the summer season, seven others were similarly treated during the winter months. This arrangement was considered advisable in order to obviate the

TABLE I

Underfed Guinea Pigs

No. of guinea pig	Original weight	Final weight	Loss of weight in grams	Per cent of loss of weight	Number of days during which underfeeding took place	Mitoses
904.....	365	248	117	32	11	0
903.....	380	275	105	27.6	11	0
902.....	395	280	115	29.1	11	120
905.....	400	290	110	27.5	11	0
400.....	380	295	85	22.4	10	0
401.....	390	275	115	29.5	10	0
402.....	405	290	115	28.4	10	0
403.....	380	300	80	21.	10	0
460.....	425	310	115	27.	9	0
461.....	410	325	85	20.7	9	0
462.....	395	295	100	25.3	9	0
463.....	405	300	105	25.9	9	0

influence of possible seasonal variations that might take place in thyroid activity. The underfeeding extended over a period of nine to eleven days; during this time the animals received the usual kind of food consisting of green vegetables and grain, but the quantity of food given each guinea pig was diminished in such a way that the animals subjected to this ration lost between 20 and 32 per cent of their original body weight. When this was accomplished the guinea pigs were killed with chloroform, the thyroids were at once removed and fixed and subsequently cut into complete serial sections. The number of mitoses in the entire gland were then estimated by means of the method described by us in a previous communication, according to which mitotic counts were made every tenth section.

The results we obtained may be briefly summarized as follows: the most striking feature in these experiments was the almost complete cessation of cell proliferation in practically every case (see Table I).

We thus found that out of the twelve underfed guinea pigs eleven failed to show any mitoses at all in the thyroid gland. In the one remaining animal we found the number of mitoses in the entire thyroid to be 120, which number is slightly below the average (see Table II).

We may therefore conclude that diminution in the amount of food intake has a very marked depressive effect on the proliferative activ-

TABLE II

Controls

No. of guinea pig	Original weight	Final weight	Gain of weight in grams	Per cent of gain of weight	Number of days during which gain in weight took place	Mitoses
259.....	368	385	17	4.6	10	150
265.....	318	348	30	9.4	10	440
337.....	470	525	55	11.7	10	264
342.....	495	570	75	15.1	10	170
Average of mitoses						256

ity of the thyroid epithelium, as evidenced by the absence of mitoses in the gland in almost all the animals examined.

As to the mechanism concerned in this process we may assume that the withdrawal of food from the animals lowers their metabolism in general as well as the metabolism of the thyroid gland, and in consequence of this effect the acinus cells cease to divide. The gland then enters a resting stage in which all activities come to a standstill.

The morphological character of the gland under these conditions confirms the conclusion that as a result of underfeeding its function is much diminished. In such a gland we find the acini reduced in size. These small acini are closely crowded together and thus the whole gland appears very cellular. The epithelium lining the acini is generally cuboidal and whereas in certain places it is flattened out, in other places it may be somewhat higher. The nuclei usually stain deeply with hematoxylin and appear granular. The colloid is very hard and fills the entire lumen of the acinus; the slight retraction of the colloid from the acinus cells which is usually found in a normal gland is lacking. Vacuole formation in the periphery of the colloid,

or other signs of liquefaction and absorption of the colloid is not usually observed in these glands. Furthermore, phagocytic activity which is so marked a feature in animals fed with KI, and which occurs likewise, although to a less extent in normal thyroids,[6] is practically absent in the colloid filling the acini of underfed guinea pigs. The structural condition of the gland corresponds therefore to that of a resting, not actively functioning gland.

In the light of these experimental findings we may conclude that the structural and proliferative conditions in the thyroid are influenced by the general body metabolism. Secondarily, the changes that take place in this gland must influence the metabolic energy. It is known that an increased thyroid activity is usually followed by an increased metabolism, and a decreased thyroid activity by a decreased metabolism. We now find that a diminished metabolism decreases the activity of the thyroid gland so that it approaches a resting stage. Under these conditions it is reasonable to assume that the reduction in general metabolism which occurs in case of general undernutrition is in part due to, or at least is intensified by the changes which take place in the thyroid gland as a result of underfeeding.

Summary

1. An experimental study was made to determine the effects of underfeeding on the thyroid activity in guinea pigs.

2. When young guinea pigs following underfeeding extending over a period of ten to eleven days lose from 20 to 32 per cent of their body weight, the acinar epithelium of the thyroid gland ceases to proliferate, as is evidenced by the entire absence of mitoses; the colloid becomes very solid and the acini small. The thyroid assumes therefore the appearance of a resting, not actively functioning gland. We may assume that this change in the character of the thyroid gland must still further reduce the general metabolism.

REFERENCES

1. Loeb, Leo. *J. Med. Res.*, 1919, xl, 199; 1920, xli, 283.
2. Loeb, Leo. *Biol. Bull.*, 1917, xxxiii, 91.
3. Jackson, C. M. *Am. J. Anat.*, 1916, xix, 305.
4. Jackson, C. M. The Effects of Inanition and Malnutrition upon Growth and Structure. Philadelphia, P. Blakiston's Son & Co., 1925.
5. Morgulis, S. Fasting and Undernutrition. New York, Dutton, 1923.
6. Gray, S. H., and Loeb, Leo. *Am. J. Path.*, 1928, iv, 257.
7. Rabinovitch, Jacob. *Am. J. Path.*, 1928, iv, 601.

THE EFFECT OF INTRAPERITONEAL INJECTION OF POTASSIUM IODIDE ON THE PROLIFERATIVE ACTIVITY OF THE THYROID GLAND IN GUINEA PIGS *

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In a previous communication [1] we have shown that the feeding of potassium iodide to normal guinea pigs for a period of two to three weeks results in a very pronounced increase in the mitotic proliferation of the thyroid epithelium, an increase which was even greater than that previously observed by Gray and Loeb.[2] Furthermore, we determined that there is a definite relation between the amount of KI fed and the increase in proliferative activity. Previously Loeb [3] had shown that feeding of KI to guinea pigs in which the greater part of the thyroid gland had been removed did not prevent compensatory hypertrophy, but on the average tended to increase the latter. Marine [4] on the other hand, maintains that the intraperitoneal injection of KI to guinea pigs previously subjected to partial thyroidectomy prevents the onset of compensatory hypertrophy. Since Marine did not feed KI to his guinea pigs but made use of intraperitoneal injections, it was thought possible that the differences in the results obtained by Marine and other investigators were due perhaps to the different methods of administration of iodide; we thought it therefore advisable to carry out another series of experiments in which, instead of feeding potassium iodide we injected it intraperitoneally. It was conceivable that the more rapid absorption of KI which takes place if the latter method is used might produce certain quantitative changes in the results obtained, and this possibility added to the interest of the experiment.

OUTLINE OF EXPERIMENTS

In this investigation four sets of guinea pigs were selected three of which were injected intraperitoneally with varying quantities of

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potassium iodide for different periods of time, while the fourth served as a control group. In the first set each animal received 0.01 gm. KI, in the second set 0.05 gm. KI, and in the third set 0.1 gm. KI, daily. The fourth set did not receive any potassium iodide. The potassium iodide to be injected was in each case dissolved in 0.5 cc. of sterile distilled water. This course of treatment was continued for periods of ten, fifteen, twenty and thirty days; at the end of each of these periods the thyroids were removed and studied in the manner described by us in our previous paper.[1] The weight of the guinea pigs used in these experiments varied between 350 and 500 gm.

Counts of Mitoses

Control Animals: The number of mitoses in the thyroid glands of the control animals varied between 136 and 280, the average being 212.

Animals Receiving Intraperitoneal Injections of KI for Ten Days: Animals that received intraperitoneal injections of KI for a period of ten days showed a considerable increase in the number of mitoses. In accordance with our previous findings this increase was greater, the greater the dose of KI administered. We thus found that the animal which received 0.01 gm. KI had 1050 mitoses, the one receiving 0 05 gm. KI, 2370 mitoses, while the guinea pig receiving 0.1 gm. had 4620 mitoses in the thyroid gland.

It is apparent from these figures that the intraperitoneal injection of KI for a period of ten days causes a marked proliferation of the thyroid epithelium, and furthermore that the larger the dose of iodide injected the more intense is the resulting mitotic cell proliferation. In our previous experiments in which the iodide had been given by mouth, the stimulating action of KI during the first ten days was very slight and it was not until the fifteenth or twentieth day that the marked stimulating influence of the substance was observed. It appears therefore that the route by which iodide is administered plays an important rôle in the rapidity with which the effects of this substance on the thyroid gland become noticeable, and that intraperitoneal injection acts more expeditiously than feeding by mouth. The more rapid absorption of the iodide when injected intraperitoneally is probably responsible for the effect at an earlier period.

Animals Injected with KI for a Period of Fifteen Days: In this group the number of mitoses in animals receiving 0.01, 0.05, and 0.1 gm. KI was 1040, 2828 and 2150 respectively. The counts were about the same as those obtained after ten days in the animals receiving 0.01 gm. KI; it was slightly increased after fifteen days in the one receiving 0.05 gm. KI, and was decidedly decreased in the last one receiving 0.1 gm. KI.

As compared with corresponding guinea pigs examined after ten days we may conclude that on the whole after fifteen days of KI injection the mitotic proliferation is still much increased over that found in the controls, but that it does not exceed the intensity found after ten days; it may even be somewhat lower. It may be suggested as probable that during the first ten days of the injection with KI the animal becomes more or less saturated with the substance and that in case of continued injection the response of the gland to the stimulating effects of the iodide becomes less.

Animals Injected with KI for a Period of Twenty Days: When the injection of KI is continued for twenty days there results a marked reduction in cell proliferation as compared with that of the shorter periods of iodide administration. The mitotic count however, is still higher than that found in the control group. Thus in animals receiving 0.01, 0.05, and 0.1 gm. KI the number of mitoses was 350, 470 and 840 respectively. In this period the response of the thyroid to the stimulating substance has become less.

Animals Injected with KI for a Period of Thirty Days: The number of mitoses in this group of cases was 520, 1120, and 1360 in animals receiving 0.01, 0.05 and 0.1 gm. KI respectively. We see then that there is still an increase in mitotic activity at this time over that noted in the control animals, and this increase is even somewhat greater than in the preceding period of twenty days. However, the difference between the counts found at twenty and thirty days is relatively small and further investigation must show whether it is a chance phenomenon, perhaps connected with the somewhat greater increase in weight of the animals of this group.

At present we can merely draw the conclusion that in animals injected intraperitoneally with KI for thirty days there is still an increased mitotic activity of the gland, whereas in our former investigations we have found that in guinea pigs fed with this substance for the same period of time all proliferation has ceased. Whether

this difference is due to differences in the mode of administration of KI used in these two series, or whether it is due to some secondary factor likewise needs further investigation. For a comparison of the number of mitoses found in the controls and in the injected animals at the various periods of time we refer to the table inserted below.

TABLE OF MITOSES *

Time in days	Control	0.01 gm. KI	0.05 gm. KI	0.1 gm. KI
10.....	264 (55+)	1050 (35+)	2370 (20+)	4620 (2−)
15.....	170 (75+)	1040 (130+)	2828 (100+)	2150 (5+)
20.....	136 (140+)	350 (98+)	470 (50+)	840 (5+)
30.....	280 (245+)	520 (115+)	1120 (130+)	1360 (38+)
Averages	212	740	1697	2242

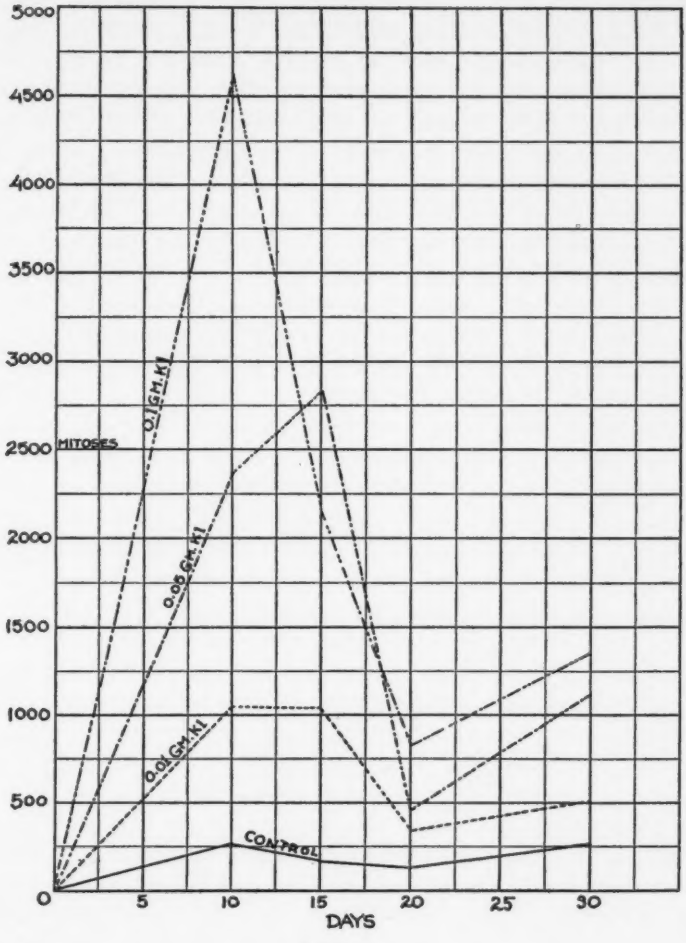
* The figures in brackets indicate the gain or loss in body weight. + = gain in weight; − = loss in weight.

DISCUSSION

The question now arises as to the cause of the difference in effects on the thyroid of the oral administration of KI and of the intraperitoneal injection. The ready absorption of the iodide from the peritoneal cavity and its consequent earlier withdrawal from the blood by the thyroid might lead to a more rapid accumulation of this substance in the gland, the stimulation of the gland tissue resulting therefrom; also such a stimulation if continued for fifteen, twenty or thirty days, becomes less effective and the thyroid therefore reacts more sluggishly; thus the excessive proliferative activity is diminished.

The changes in proliferative activity of the thyroid gland of guinea pigs under the influence of intraperitoneal injection of potassium iodide, are represented in the curves of the attached chart in which in each case the number of days during which injections were given is the abscissa and the number of mitoses, the ordinate. If we compare this chart with the one in our preceding paper representing the variations in proliferative activity in the thyroid gland caused by oral administration of potassium iodide, we find some interesting differences. After feeding this substance the curves were relatively low during the first ten days but rose abruptly at fifteen days, reach-

ing the peak at the end of the twentieth day and falling again to normal, or even to subnormal levels at the end of the thirtieth day. After intraperitoneal injection of the iodide, the curves rise very



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much earlier and reach the peak at the end of the tenth day. From then on until the fifteenth day they either fall, hold their own, or show a slight rise. They all decline abruptly between the fifteenth

and twentieth day without however reaching the base line, and they remain still somewhat elevated after thirty days. There has even been a very slight rise between the twentieth and thirtieth day which may however be without significance.

In this series of experiments we find on the whole within the range of our experimental variations, that the increase in proliferative activity is the greater the larger the quantity of potassium iodide given. Only at fifteen days the proliferation is slightly less in the guinea pig receiving 0.1 gm. daily than in the guinea pig receiving 0.05 gm. of KI. We must however take into consideration the fact that the animals injected with the largest doses of KI did not gain in weight except the ones examined at thirty days when a slight gain was noticed, whereas the guinea pigs injected with the smaller doses all gained in weight. The daily intraperitoneal injection of as much as 0.1 gm. of KI into a guinea pig may influence the weight in this way by leading to an excess mobilization of thyroxin or by some other as yet undetermined process.

Structural Changes in Acini, Epithelium and Colloid: In the control series the acini are on the average of small or medium size lined with cuboidal epithelium and filled with solid colloid. We also find a moderate number of phagocytes in the colloid of some of the various acini. The microscopic appearance of the thyroid in animals injected with potassium iodide does not differ to any appreciable degree from that noted in the controls; the alveoli in the former are on the average of medium size, although they occasionally appear somewhat larger. The epithelium is cuboidal or columnar and the colloid is still solid but not to the same extent as in the control group. In the thyroid of animals injected with KI softening processes in the colloid are more marked, and correspondingly the number of phagocytes in the colloid is on the average greater than in the controls. However the watery, pale colloid that we noted in our previous experiments after thirty days of feeding of potassium iodide is entirely lacking in the injected guinea pigs. We also fail to find here the flattening of the lining epithelium and the consequent fusion and coalescence of neighboring acini which we noted at this period after oral administration of iodide.

Summary

1. The intraperitoneal injection of KI for a period of ten days causes a very rapid increase in proliferation of the thyroid epithelium, as evidenced by the great increase in the number of mitoses observed at this time. Between the tenth and fifteenth day the proliferative activity remains about equally high, except in the animal receiving the largest dose of KI where a decrease was noticed; it is considerably lower after twenty and thirty days of injection, but is still increased as compared with the number of mitoses found in the control animals.

2. In case of intraperitoneal injection of potassium iodide the average increase in proliferative activity in the thyroid gland is the greater the larger the amount of KI administered, within the range of the doses used by us. The curves representing the changes in proliferative activity of these animals, show, therefore, a characteristic difference from that representing the corresponding changes in guinea pigs fed with potassium iodide. These differences are due to differences in the rapidity of absorption and in the gradients of concentration of KI established between the blood and the thyroid gland and the organs through which elimination occurs.

3. The structural appearance of the thyroid of animals injected with KI does not differ very markedly from that of the controls. However, occasionally a slight softening of the colloid and a more active phagocytosis may be found in the injected animals.

REFERENCES

1. Rabinovitch, Jacob. *Am. J. Path.*, 1928, iv, 601.
2. Gray, S. H., and Loeb, Leo. *Am. J. Path.*, 1928, iv, 257.
3. Loeb, Leo. *J. Med. Res.*, 1919, xl, 199; 1920, xli, 481; 1920, xlii, 77. *Am. J. Path.*, 1926, ii, 19.
4. Marine, David. *Arch. Path. & Lab. Med.*, 1926, ii, 829.